DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR QUANTIFICATION OF TAPENTADOL HYDROCHLORIDE IN TABLET DOSAGE FORM

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KMCH COLLEGE OF PHARMACY

KOVAI ESTATE, KALAPPATTI ROAD,

COIMBATORE- 641048.

Dr. A. Rajasekaran M.Pharm, Ph.D.,
Principal,
K.M.C.H. College of Pharmacy,
Kovai Estate, Kalapatti Road,
Coimbatore-641048

CERTIFICATE

This is to certify that the dissertation work entitled **"DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR QUANTIFICATION OF TAPENTADOL HYDROCHLORIDE IN TABLET DOSAGE FORM"** is a bonafide research work carried out by **Mr. VENKANA BABU. MADETI (Reg. No: 26107229)** , student in Master of Pharmacy, Department of Pharmaceutical Analysis, K.M.C.H.College of Pharmacy, Coimbatore, Tamilnadu, under the guidance of **Mr. J.DHARUMAN**, **Professor**, Head, Department of Pharmaceutical Analysis, K.M.C.H. College of Pharmacy during the academic year 2011-2012.

I wish for him best career,

Signature

Date: **Dr. A.Rajasekaran M.Pharm, Ph.D.**

Place: Principal

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INTRODUCTION

Analytical chemistry [1] is often described as the area of chemistry responsible for characterizing the composition of matter, both qualitatively (what is present) and quantitatively (how much is present).Analytical chemistry is not a separate branch of chemistry, but simply the application of chemical knowledge.

Pharmaceutical Analysis [2] is the branch of chemistry involved in separating, identifying and determining the relative amounts of the components making up a sample of matter. It is mainly involved in the qualitative identification or detection of compounds and quantitative measurements of the substances present in bulk and pharmaceutical preparation.

Quantitative analysis constitutes the largest part of analytical chemistry and is related to the various methods and instrumentation employed in determining the amounts or concentration of constituents in samples. It is also one of the basic criteria in the field of pharmacy where quality is to be critically maintained. Analytical chemistry may be defined as the "Science and art of determining the composition of materials in terms of the elements or compounds contained". Analytical method is a specific application of a technique to solve an analytical problem [3].

Physico-chemical methods [4, 5] are used to study the physical phenomenon that occurs as a result of chemical reactions. Among the Physico-chemical methods, the most important are optical (Refractometry, Polarimetry, Emission, Fluorescence methods of analysis, Photometry including Photo-colorimetry and Spectrophotometry covering UV-Visible and IR regions and Nephelometry or Turbidimetry) and chromatographic (Column, Paper, TLC, GLC, HPLC) methods. Methods such as Nuclear Magnetic Resonance and Para Magnetic Resonance are becoming more and more popular. The combination of Mass Spectroscopy with Gas

Chromatography and Liquid Chromatography are the most powerful tools available. The chemical methods include the gravimetric and volumetric procedures which are based on complex formation; acid-base, precipitation and redox reactions. Titrations in non-aqueous media and complexometry have also been used in pharmaceutical analysis.

The number of new drugs is constantly growing. This requires new methods for controlling their quality. Modern pharmaceutical analysis must need the following requirements.

I. The analysis should take a minimal time.
II. The accuracy of the analysis should meet the demands of Pharmacopoeia.
III. The analysis should be economical.
IV. The selected method should be precise and selective.

These requirements are met by the Physico-chemical methods of analysis, a merit of which is their universal nature that can be employed for analyzing organic compounds with a diverse structure. Of them, Visible Spectrophotometry is generally preferred especially by small scale industries as the cost of the equipment is less and the maintenance problems are minimal.

Pharmaceutical analysis techniques are applied mainly in two areas [6]:

Traditionally, analytical chemistry has been split into two main types, Qualitative and

Quantitative

1. **Qualitative:** Qualitative analysis seeks to establish the presence of a given element or compound in a sample.

2. **Quantitative:** Quantitative analysis seeks to establish the amount of a given element or compound in a sample.

Instrumental methods of Chemical analysis:

Instrumental method is an exciting and fascinating part of chemical analysis that interacts with all areas of chemistry and with many other areas of pure and applied sciences. Analytical instrumentation plays an important role in the production and evaluation of new products and in the protection of consumers and environment. This instrumentation provides lower detection limits required to assure safe foods, drugs, water and air. Instrumental methods are widely used by analytical chemists to save time, to avoid chemical separation and to obtain increased accuracy. Different types of techniques are listed below:

A) Spectrometric techniques:

- Atomic Spectrometry (emission and absorption)
- Electron Spin Resonance Spectroscopy
- Fluorescence and Phosphorescence Spectrophotometry
- Infrared Spectrophotometry
- Nuclear Magnetic Resonance Spectroscopy
- Radiochemical Techniques including activation analysis
- Raman Spectroscopy
- Ultraviolet and Visible Spectrophotometry
- X-Ray Spectroscopy

B) Chromatographic Techniques:

- Gas Chromatography
- High Performance Liquid Chromatography
- Thin Layer Chromatography

C) Miscellaneous Techniques:

- Kinetic Techniques
- Mass Spectrometry
- Thermal Analysis

D) Hyphenated Techniques:

- GC-MS (Gas Chromatography – Mass Spectrometry).
- ICP-MS (Inductivity Coupled Plasma- Mass Spectrometry).
- GC-IR (Gas Chromatography – Infrared Spectroscopy).
- MS-MS (Mass Spectrometry – Mass Spectrometry).

INTRODUCTION TO CHROMATOGRAPHY [7]:

Chromatography, by classical definition, is a separation process where resolution is achieved by the distribution of the components of a mixture between two phases, a stationary phase and a mobile phase. Those components held preferentially in the stationary phase are retained longer in the system than those that are distributed in the mobile phase. As a consequence solutes are eluted from the system in the order of their increasing distribution coefficients with respect to the stationary phase. The mobile phase can be a gas or a liquid which gives rise to the two basic forms of Chromatography, namely, Gas Chromatography (GC) and Liquid Chromatography (LC). The stationary phase can also take two forms, solid and liquid, which provides two subgroups of GC and LC, namely; Gas-Solid Chromatography (GSC) and Gas-Liquid Chromatography (GLC), together with Liquid Solid Chromatography (LSC) and Liquid- Liquid Chromatography (LLC).

INTRODUCTION -HIGH PERFORMANCE LIQUID CHROMATOGRAPHY [8, 9]:

In the modern pharmaceutical industry, HPLC is a major analytical tool applied at all stages of drug discovery, development and production. Fast and effective development of rugged analytical HPLC methods is more efficiently undertaken with a thorough understanding of HPLC principles, theory and instrumentation.

Principle [10]:

The principle of separation in normal phase mode and reverse phase mode is adsorption. When mixtures of components are introduced in to a HPLC column, they travel according to their relative affinities towards the stationary phase. The component which has more affinity towards the adsorbent travels slower.

Types of HPLC [11]:

There are many ways to classify Liquid Column Chromatography. If classification is based on the nature of the stationary phase and the separation process,

Three modes can be specified:

In **Adsorption Chromatography:**

The stationary phase is an adsorbent (like silica gel or any other silica based packing) and the separation is based on repeated adsorption desorption steps.

In **Ion-Exchange Chromatography:**

1. The stationary bed has an ionically charged surface of opposite charge to the sample ions.

2. This technique is used almost exclusively with ionic or ionizable samples.

3. The stronger the charge on the sample, the stronger it will be attracted to the ionic surface and thus, the longer it will take to elute.

4. The mobile phase is an aqueous buffer, where both pH and ionic strength are used to control elution time.

In **Size Exclusion Chromatography:**

1. The column is filled with material having precisely controlled pore sizes, and the sample is simply screened or filtered according to its solvated molecular size.

2. Larger molecules are rapidly washed through the column; smaller molecules penetrate inside the porous of the packing particles and elute later.

3. Concerning the first type, two modes are defined depending on the relative polarity of the two phases: Normal and Reversed-Phase Chromatography.

In **Normal-Phase Chromatography**:

1. The stationary bed is strongly polar in nature (e.g., silica gel), and the mobile phase is non-polar (such as n-hexane or tetrahydrofuran).

2. Polar samples are thus retained on the polar surface of the column packing longer than less polar materials.

In **Reversed-Phase Chromatography:**

The stationary bed is non-polar in nature, while the mobile phase is a polar liquid, such as mixtures of Water and Methanol or Acetonitrile. Here the more non-polar the material is, the longer it will be retained. The component which has less affinity towards the stationary phase

travels faster. Since no two components have the same affinity towards the stationary phase, the components are separated.

Liquid Chromatography (LC), which is one of the forms of Chromatography, is an analytical technique that is used to separate a mixture in solution into its individual components. The separation relies on the use of two different "phases" or "immiscible layers," one of which is held stationary while the other moves over it. Liquid Chromatography is the generic name used to describe any chromatographic procedure in which the mobile phase is a liquid. The separation occurs because, under an optimum set of conditions, each component in a mixture will interact with the two phases differently relative to the other components in the mixture. HPLC is the term used to describe Liquid Chromatography in which the liquid mobile phase is mechanically pumped through a column that contains the stationary phase.

An HPLC instrument, therefore, consists of an injector, a pump, a column, and a detector given in the Fig: 1.

Liquid Chromatography has come a long way with regard to the practical development of HPLC instrumentation and the theoretical understanding of different mechanisms involved in the analyte retention as well as the development of adsorbents with different geometries and surface chemistry.

In HPLC, a liquid sample or a solid sample dissolved in a suitable solvent is carried through a Chromatographic column by a liquid mobile phase. Separation is determined by solute/stationary-phase interactions, including liquid–solid adsorption, liquid–liquid partitioning, ion exchange and size exclusion, and by solute/ mobile-phase interactions, which are listed in

Table: 1 with their applications. In each case, however, the basic instrumentations are essentially same.

Table no: 1 Various Types of HPLC

TYPE	SAMPLE POLARITY	MOLECULAR WEIGHTRANGE	STATIONARY PHASE	MOBILE PHASE
Adsorption	non-polar to somewhat polar	100– 104	silica or alumina	non-polar to polar
Partition (reversed-phase)	non-polar to somewhat polar	100– 104	non-polar liquid adsorbed or chemically bonded to the packing material	relatively polar
Partition (normal-phase)	somewhat polar to highly polar	100– 104	highly polar liquid adsorbed or chemically bonded to the packing material	relatively non-polar
Ion Exchange	highly polar to ionic	100 – 104	ion-exchange resins made of insoluble, high-molecular weight solids functionalized typically with sulfonic acid (cationic exchange) or amine (anionic exchange) groups	aqueous buffers with added organic solvents to moderate solvent strength
Size-Exclusion	non-polar to ionic	103 – 106	small, porous, silica or polymeric particles	polar to non-polar

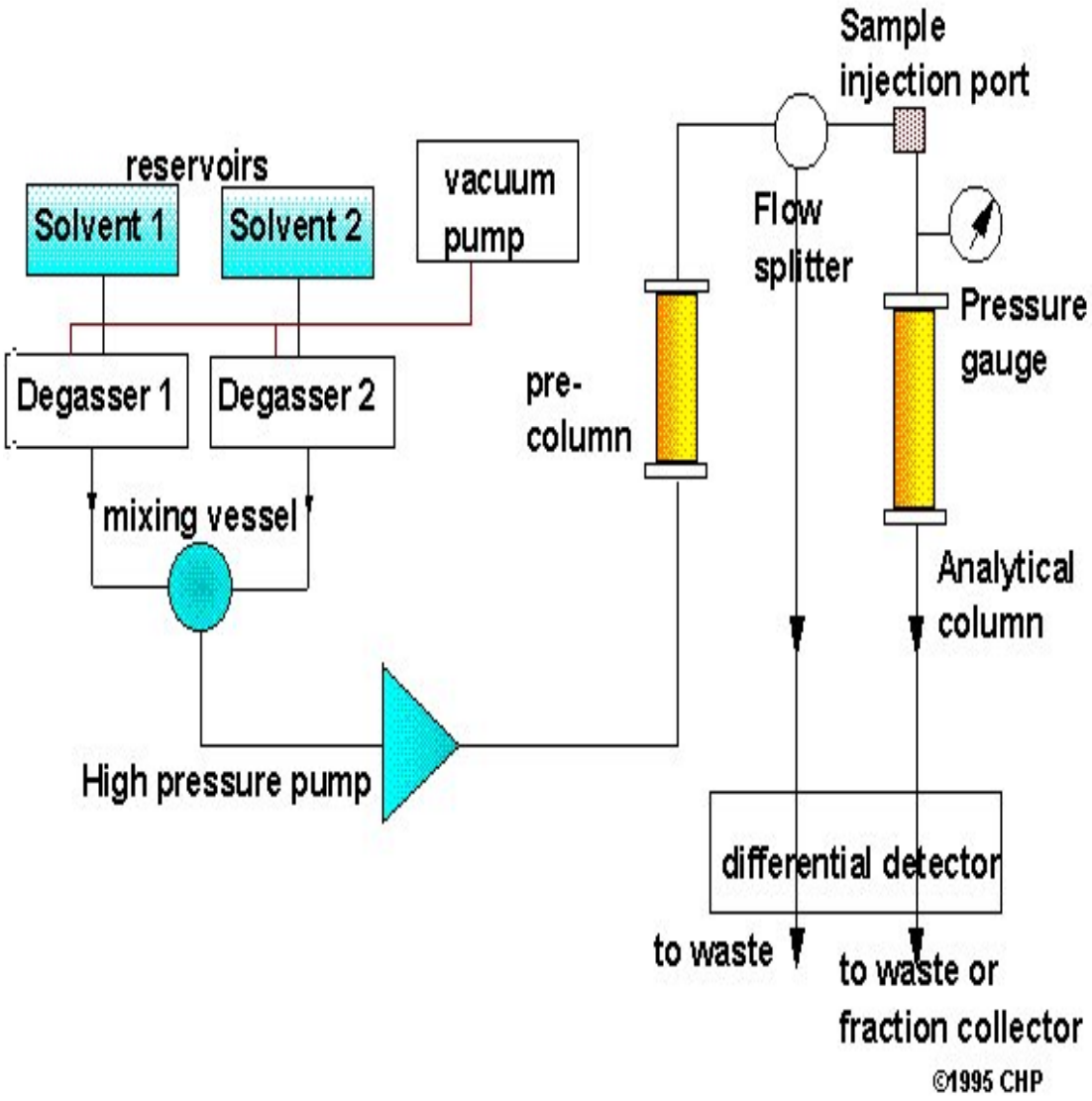


Fig: 1 HPLC BASIC INSTRUMENT [12]

Various components involved in HPLC:

1. **Solvent delivery systems**:

The purpose of the pump, or solvent delivery system, is to ensure the delivery of a precise, reproducible, constant, and pulse-free flow of mobile phase.

There are two classes of HPLC pumps:

1. Constant pressure pumps

2. Constant flow pumps

The most common type of HPLC constant flow pump is the reciprocating piston pump, in which a piston is driven in and out of the solvent chamber by a gear. On the forward stroke, the inlet check valve closes, the outlet check valve opens, and the mobile phase is pumped to the column. On the return stroke, the check valves reverse and solvent is drawn into the chamber. In the single head reciprocating pump, 50 % of the time the mobile phase flows to the column and 50 % of time the chamber is refilling. With the twin-head reciprocating pump the pump heads operate simultaneously but 1800 out of phase with each other. As a result mobile phase flows to the column 100 % of the time, providing an essentially pulse less flow.

Most separations can be done using isocratic elution which is the use of a single-solvent system that does not change during the analysis. For more complex analysis gradient elution is required. Gradient elution can be generated in three ways. In all cases a computer controlled pumping system is required. In the first phase, controlled amounts of each eluent are metered into a mixing chamber before reaching the high-pressure pump which sends the mixture to the column. In second case the amount of each solvent is regulated by a proportionating valve which is

controlled by a microprocessor. The mixed solvent then enters the high pressure pump and flows to the column. In the third case the delivery of high delivery multiple pumps is controlled individually with a programming device and mixer is send to high-pressure mixing chamber

2. Columns [13]: The heart of the system is the column. The choice of common packing material and mobile phases depend on the physical properties of the drug. The column selection flow chart can assist one in determining which columns to examine. Many different reverse phase columns will provide excellent specificity for any particular separation. It is therefore best to routinely attempt separations with a standard C-8 or C-18 column (e.g. Zorbax RX C-8) and determine if it provides good separations. If this column does not provide good separation or the mobile phase is unsatisfactory, alternate methods or columns should be explored. Reverse phase column differ by the carbon chain lengths, degree of end capping and percent carbon loading. Diol, Cyano and amino groups can be used for Reverse Phase Chromatography.The most commonly used columns for HPLC are constructed from stainless steel with internal diameters between 2.1 mm and 4.6 mm, and lengths ranging from approximately 30 mm to 300 mm. These columns are packed with 3–10 mm porous silica particles that may have an irregular or spherical shape. Typical column efficiencies are 40,000–60,000 theoretical plates/m. Micro columns use less solvent and, because the sample is diluted to a lesser extent, produce larger signals at the detector. These columns are made from fused silica capillaries with internal diameters of 44–200 mm and lengths of up to several meters. Micro columns packed with 3–5 mm particles have been prepared with column efficiencies of up to 250,000 theoretical plates. Open tubular micro columns also have been developed, with internal diameters of1–50 mm and lengths of approximately 1 m. These columns, which contain no packing material, may be capable of obtaining column efficiencies of up to 1 million theoretical plates. The development of open

tubular columns, however, has been limited by the difficulty of preparing columns with internal diameters less than 10 mm.

3. Stationary Phases [14]:

In Liquid–Liquid Chromatography the stationary phase is a liquid film coated on a packing material consisting of 3–10 mm porous silica particles. The stationary phase may be partially soluble in the mobile phase, causing it to "bleed" from the column over time. To prevent this loss of stationary phase, it is covalently bound to the silica particles. Bonded stationary phases are attached by reacting the silica particles with an organochlorosilane of the general form Si $(CH_3)2RCl$, where R is an alkyl or substituted alkyl group. To prevent unwanted interactions between the solutes and any unreacted –SiOH groups, the silica frequently is "capped" by reacting it with Si $(CH_3)3Cl$; such columns are designated as end-capped. The properties of a stationary phase are determined by the nature of theorganosilane's alkyl group. If R is a polar functional group, then the stationary phase will be polar. Since the stationary phase is polar, the mobile phase is a non-polar or moderately polar solvent. The combination of a polar stationary phase and a non-polar mobile phase is called normal phase Chromatography.

In reverse phase Chromatography, which is the more commonly encountered form of HPLC, the stationary phase is non-polar and the mobile phase is polar. The most common non-polar stationary phases use an organochlorosilane for which the R group is an n-octyl (C8) or n-octyldecyl (C18) hydrocarbon chain. Most reverse phase separations are carried out using a buffered aqueous solution as a polar mobile phase.

4. Mobile Phases [15]

The elution order of solutes in HPLC is governed by polarity. In a normal-phase separation the least polar solute spends proportionally less time in the polar stationary phase and is the first solute to elute from the column.

The mobile phases used in normal-phase chromatography are based on non-polar hydrocarbons, such as hexane, heptane, or octane, to which is added a small amount of a more polar solvent, such as 2-propanol. Solvent selectivity is controlled by the nature of the added solvent. Additives with large dipole moments, such as methylene chloride and 1, 2-dichloroethane, interact preferentially with solutes that have large dipole moments, such as nitro-compounds, nitriles, amines, and sulfoxides. Good proton donors such as Chloroform, m-Cresol, and Water interact preferentially with basic solutes such as amines and Sulfoxides, whereas good proton acceptors such as alcohols, ethers, and amines tend to interact best with hydroxylated molecules such as acids and phenols. A variety of solvents used as mobile phases in normal-phase Chromatography are listed in Table: 1.2, some of which may need to be stabilized by addition of an antioxidant, such as 3-5 % Ethanol, because of the propensity for peroxide formation.

In a reverse-phase separation the order of elution is reversed, with the most polar solute being the first to elute. The mobile phases used in reversed-phase Chromatography are based on a polar solvent, typically water, to which a less polar solvent such as Acetonitrile or Methanol is added. Solvents with large dipole moments, such as methylene chloride and 1,2-dichloroethane, interact preferentially with solutes that have large dipole moments, such as nitro- compounds, nitriles, amines, and sulfoxides. Solvents that are good proton donors, such as Chloroform, m-Cresol, and Water, interact preferentially with basic solutes such as amines and Sulfoxides, and solvents that

are good proton acceptors, such as alcohols, ethers, and amines tend to interact best with hydroxylated molecules such as acids and phenols.

Table: 2 List of solvents used in HPLC

Solvent	Adsorption energy(e0) on Al_2O_3	Solvent	Adsorption energy(e0) on Al_2O_3
n-Pentane	0.00	Acetone	0.56
Isooctane	0.01	Ethyl Acetate	0.58
Cyclohexane	0.04	Dimethylamine	0.63
Carbon Tetrachloride	0.18	Acetonitrile	0.65
Toluene	0.29	Ethanol	0.88
Benzene	0.32	Methanol	0.95
Chloroform	0.40	Acetic Acid	Large
Methyl Ethyl Ketone	0.51	Water	Very large

5. Detectors [16]

The detection of UV light absorbance offers both convenience and sensitivity for molecules. When a chromophore, the wavelength of detection for a drug should be based on its UV spectrum in the mobile phase and not in pure solvents, the most selective wavelength for detecting a drug is frequently the longest wavelength maximum to avoid interference from solvents, buffers and excipients. Other method of detection can be useful are required in some instances.

- Solute specific detectors (UV, Visible, Fluorescence, Electrochemical, IR, Radioactivity).
- Bulk property detectors (Refractive index, Viscometric, Conductivity).
- Desolvation detectors (Flame ionization etc.).

- LC-MS detectors.
- Reaction detectors.

SYSTEM SUITABILITY PARAMETERS [17]

System suitability tests are an integral part of Gas and Liquid Chromatography. They are used to verify that the resolution and reproducibility of the Chromatographic system are adequate for the analysis to be done. These tests are based on the concept that the equipment, electronics, analytical operations and samples to be analyzed constitute an integral system that can be evaluated as such.

There are numerous guidelines which detail the expected limits for typical Chromatographic methods. In the current FDA guideline on "Validation of Chromatographic methods" the following acceptance limits are proposed as initial criteria.

1. Capacity factor (k'):

$k' = (t_R - t_0) / t_0$

The capacity factor is a measure of the degree of retention of an analyte relative to an unretained peak.

Where, t_R- retention time for the sample peak.

t_0- retention time for the unretained peak.

The peak should be well-resolved from other peaks and the void volume. Generally the value of k' is > 2.

2. Backpressure:

The pressure required to pump the mobile phase through the column. It is related to mobile phase viscosity (η), flow rate (F), column length (L), and diameter (dc), and particle size (dp) by the following equation:

$$\Delta P \alpha FL\eta / dp2\ dc2$$

3. Resolution (Rs):

Ability of a column to separate chromatographic peaks, Resolution can be improved by increasing column length, decreasing particle size, increasing temperature, changing the eluent or stationary phase. It can also be expressed in terms of the separation of the apex of two peaks divided by the tangential width average of the peaks.

$Rs = \Delta tR / 0.5\ (W1 + W2);$

Where, $\Delta tR = t2 - t1$.

For reliable quantization, well-separated peaks are essential for quantitation. Rs of > 2 between the peak of interest and the closest potential interfering peak (impurity, excipients, degradation product, internal standard, etc.) are desirable.

4. Theoretical plate number / Efficiency (N):

A measure of peak band spreading determined by various methods, some of which are sensitive to peak asymmetry. The most common are shown here, with the ones most sensitive to peak shape shown first.

4-sigma / tangential

N = 16 (tR / W) 2 = L / H

Half height

N = 5.54(tR/ W) 2 = L / H

Theoretical plate number is a measure of column efficiency, that is, how many peaks can be located per unit run-time of the chromatogram.

Where, tR - Retention time for the sample peak.

W - Peak width.

N is fairly constant for each peak on a chromatogram with a fixed set of operating conditions. H (height), or HETP (height equivalent of a theoretical plate), measures the column efficiency per unit length (L) of the column. Parameters which can affect N or H include.

Peak position, particle size in column, flow-rate of mobile phase, column temperature, viscosity of mobile phase, and molecular weight of the analyte.

The theoretical plate number depends on elution time but in general should be > 2000.

5. Tailing factor (T):

It is the measure of the symmetry of a peak.

T = W0.05 / 2f

Where, W0.05 - Peak width at 5% height

F - Distance from peak front to apex point at 5% height.

The accuracy of quantitation decreases with increase in peak tailing because of the difficulties encountered by the integrator in determining where/when the peak ends and hence the calculation of the area under the peak.

Limits -T</= 2.

Advantages of HPLC [18]**:**

The HPLC is the method of choice in the field of analytical chemistry, since this method is Specific, Robust, Linear, Precise and Accurate and the Limit of detection is low and also it offers the following advantages.

1. Speed (many analysis can be accomplished in 20 min or less).

2. Greater sensitivity (various detectors can be employed).

3. Improved resolution (wide variety of stationary phases).

4. Reusable columns (expensive columns but can be used for many analysis).

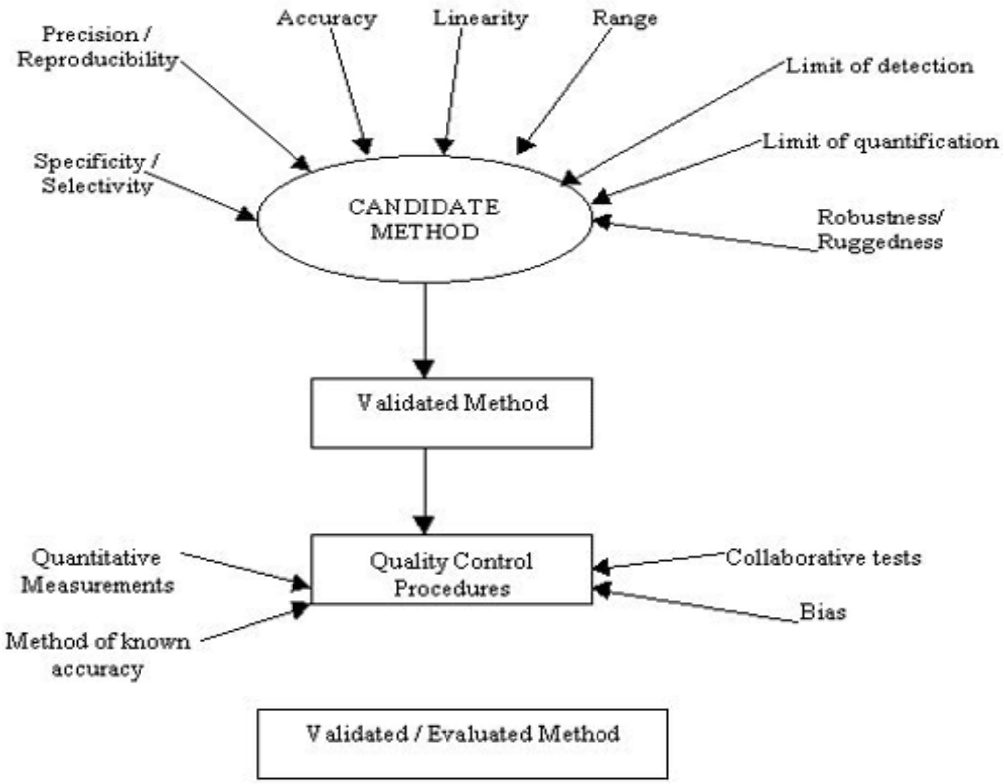
5. Easy sample recovery, handling and maintenance.

6. Instrumentation leads itself to automation and quantification (less time & less labour).

7. Precise and reproducible and integrator itself does calculations.

INTRODUCTION TO VALIDATION [19-25]**:**

Validation:



Validation of an analytical method is the process by which it is established, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications.

Validation is defined as follows by different agencies:

1. Food and Drug administration (FDA):

Establishing documentation evidence, which provides a high degree of assurance that a specific process, will consistently produce a product meeting its predetermined specifications and quality attributes.

2. World Health Organization (WHO):

Action of providing that, any procedure, process, equipment, material, activity, or system actually leads to the expected results.

3. European Committee (EC):

Action of providing in accordance with the principles of good manufacturing practice that any procedure, process, equipment, material, activity or system actually leads to the expected results. In brief validation is a key process for effective Quality Assurance.

Analytical method validation:

Method validation is the process to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Methods need to be validated or revalidated.

Before their introduction into routine use

- Whenever the conditions change for which the method has been validated, e.g., instruments with different characteristics.
- Whenever the method is changed, and the change is outside the original scope of the method. The International Conference on Harmonization (ICH) of Technical Requirements for the Registration of Pharmaceutical for human use has developed a consensus text on the validation of analytical procedures. The document includes definitions for validation characteristics.

The parameters are:

i. Precision
ii. Specificity
iii. Accuracy
iv. Linearity
v. Range
vi. Limit of Detection
vii. Limit of Quantitation
viii. Robustness
ix. Ruggedness

The parameters as defined by the ICH and by other organizations are;

Precision:

"The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels; repeatability, intermediate precision and reproducibility."

Precision should be obtained preferably using authentic samples. As parameters, the standard deviation (SD), the relative standard deviation (coefficient of variation) and the confidence interval should be calculated for each level of precision.

Repeatability expresses the analytical variability under the same operating conditions over a short interval of time (within-assay, intra-assay). At least nine determinations covering the specified range or six determinations at 100% test concentration should be performed.

Intermediate precision includes the influence of additional random effects within laboratories, according to the intended use of the procedure, for example, different days, analysts or equipment, etc.

Reproducibility, i.e., the precision between laboratories (collaborative or inter-laboratory Studies), is not required for submission, but can be taken into account for standardization of analytical procedures.

Specificity:

"Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc. Lack of specificity of an individual procedure may be compensated by other supporting analytical procedure(s)".

With respect to identification, discrimination between closely related compounds likely to be present should be demonstrated by positive and negative samples. In the case of chromatographic assay and impurity tests, available impurities/degradants can be spiked at appropriate levels to the corresponding matrix or else degraded samples can be used. For assay, it can be demonstrated that the result is unaffected by the spiked material. Impurities should be separated individually and/or from other matrix components. Specificity can also be demonstrated by verification of the result with an independent. In the case of chromatographic separation, resolution factors should be obtained for critical separation. Tests for peak homogeneity, for example, diode array detection (DAD) or mass spectrometry (MS) are recommended.

Accuracy:

"The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found".

Accuracy can be demonstrated by the following approaches:

- Inferred from precision, linearity and specificity
- Comparison of the results with those of a well characterized, independent procedure
- Application to a reference material (for drug substance)
- Recovery of drug substance spiked to placebo or drug product (for drug product).
- Recovery of the impurity spiked to drug substance or drug product (for impurities).

For the quantitative approaches, at least nine determinations across the specified range should be obtained, for example, three replicates at three concentration levels each. The percentage recovery or the difference between the mean and the accepted true value together with the confidence intervals are recommended.

It is important to use the same quantitation method (calibration model) in the accuracy studies as used in the control test procedure. Sometimes in the literature, the data from linearity studies are simply used to calculate the content of spiked samples. However, the validation linearity study is usually not identical to the calibration applied in routine analysis. Again, validation has to demonstrate the suitability of the routine analytical procedure. Deviations from the theoretical recovery values, while performing a calibration with a drug substance alone, may indicate interferences between the analyte and placebo components, incomplete extraction, etc. In such a case, the calibration should be done with a synthetic mixture of placebo and drug substance

standard. Such interferences will also be detected by comparing the linearity's of diluted drug substance and of spiked placebo, but the evaluation is more complex. In contrast, recovery studies usually concentrate directly on the working range and are simpler (but not always easy) to evaluate.

Linearity:

"The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample".

It may be demonstrated directly on the analyte, or on spiked samples using at least five concentrations over the whole working range. Besides a visual evaluation of the analyte signal as a function of the concentration, appropriate statistical calculations are recommended, such as a linear regression. The parameters slope and intercept, residual sum of squares and the coefficient of correlation should reported. A graphical presentation of the data and the residuals is recommended.

Range:

"The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity."

Limit of detection (LOD):

"The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest concentration of analyte in a sample which can be quantitatively determined with suitable precision and accuracy."

Various approaches can be applied:

Visual definition

Calculation from the signal-to-noise ratio (LOD and LOQ correspond to 3 or2 and 10 times the noise level, respectively)

Calculation from the standard deviation of the blank

Calculation from the calibration line at low concentrations

LOD; LOQ ¼ F_SDb (2.6-1)

F: factor of 3.3 and 10 for LOD and LOQ, respectively

SD: standard deviation of the blank, standard deviation of the ordinate intercept, or residual standard deviation of the linear regression

b: slope of the regression line

The estimated limits should be verified by analyzing a suitable number of samples containing the analyte at the corresponding concentrations. The LOD or LOQ and the procedure used for determination, as well as relevant chromatograms, should be reported.

Limit of Quantitation (LOQ):

The quantitation limit is the lowest level of analyte that can be accurately and precisely measured. This limit is required only for impurity methods and is determined by reducing the analyte concentration until a level is reached where the precision of the method is unacceptable. If not determined experimentally, the quantitation limit is often calculated as the analyte concentration that gives S/N = 10. An example of quantitation limit criteria is that the limit will be defined as the lowest concentration level for which an RSD 20% is obtained when an intra-assay precision study is performed.

Robustness:

According to ICH Q2A [1a] "the robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage".

Furthermore, it is stated in ICH Q2B [1b], "The evaluation of robustness should be considered during the development phase and depends on the type of procedure understudy. It should show the reliability of an analysis with respect to deliberate variations in method parameters. If measurements are susceptible to variations in analytical conditions, the analytical conditions should be suitably controlled or a precautionary statement should be included in the procedure. One consequence of the evaluation of robustness should be that a series of system suitability parameters (e.g., resolution test) is established to ensure that the validity of the analytical procedure is maintained whenever used".

Ruggedness:

"The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of conditions, such as different laboratories, different analysts, different instruments, different days, etc. Ruggedness is normally expressed as the lack of influence on test results of operational and environmental variables of the analytical method. Ruggedness is a measure of reproducibility of test results under the variation in conditions normally expected from laboratory to laboratory and from analyst to analyst". The degree of reproducibility is then evaluated by comparison of the results obtained under varied conditions with those under standard conditions.

Table no: 3 Method Validation Requirements

Method Validation Requirements	Acceptance Criteria
Precision	
• Assay repeatability	≤ 1% RSD
• Intermediate precision (Ruggedness)	≤ 2% RSD
Accuracy	
• Mean recovery per concentration	100.0% ± 2.0%
Limit of detection	
• Signal to-to-noise ratio	≥ 3:1
Limit of quantification	
• Signal to-to-noise ratio	≥ 10:1
Linearity/Range	
• Correlation coefficient	>0.99
• y-Intercept	± 10%
• Visual	Linear
Robustness	
• System suitability met	Yes
• Solution stability	± 2% change from time zero
Specificity	
• Resolution from main peak	>2 min. (retention time)

LITERATURE REVIEW

JIN Yi et al(2011) [26] developed a sensitive and rapid LC-MS/MS method to determine the concentration of tapentadol in rat plasma. **Methods** After the extraction from plasma by protein precipitation, analytes and internal standards were separated by a Diamonsil C_{18} column. Methanol-5 mmol·L^{-1} ammonium acetate-acetic acid (*V*:*V*:*V*=58:42:0.5) were used as the mobile phase. The multiple reactions monitoring (MRM) was used for quantitative determination in positive mode. The transitions were m/z: 222.2→107.1 for tapentadol ($[M+H]^+$) , *m/z*: 307.1→220.1 for fluconazol (IS, $[M+H]^+$). **Results** No significant interferences for the detection of tapentadol and fluconazol from endogenous substances in plasma were observed in the present study. The calibration curve was linear over the range of 2.0-200 μg·L^{-1} for tapentadol. The accuracy was in the range of 96.2%-97.5% and the inter-day and intra-day precision was less than 15%. **Conclusions** The LC-MS/MS method is simple, rapid and sensitive enough for the pharmacokinetic study of Tapentadol in rats

Thomas M. Tzschentke et al[27] (_)-(1*R*,2*R*)-3-(3-Dimethylamino-1-ethyl-2-methyl-propyl)-phenol hydrochloride (Tapentadol HCl) is a novel _-opioid receptor (MOR) agonist (*K*i _ 0.1 _M; relative efficacy compared with morphine 88% in a [35S]guanosine 5_-3-*O*-(thio)triphosphate binding assay) and NE reuptake inhibitor (*K*i _ 0.5 _M for synaptosomal reuptake inhibition). In vivo intracerebral microdialysis showed that tapentadol, in contrast to morphine, produces large increases in extracellular levels of NE (_450% at 10 mg/kg i.p.). Tapentadol exhibited analgesic effects in a wide range of animal models of acute and chronic pain [hot plate, tail-flick, writhing, Randall-Selitto, mustard oil colitis, chronic constriction injury (CCI), and spinal nerve ligation (SNL)], with ED50 values ranging from 8.2 to 13 mg/kg after i.p. administration in rats. Despite a 50-fold lower binding affinity to MOR, the analgesic

potency of tapentadol was only two to three times lower than that of morphine, suggesting that the dual mode of action of tapentadol may result in an opiate-sparing effect. A role of NE in the analgesic efficacy of tapentadol was directly demonstrated in the SNL model, where the analgesic effect of tapentadol was strongly reduced by theα2-adrenoceptor antagonist yohimbine but only moderately attenuated by the MOR antagonist naloxone, whereas the opposite was seen for morphine. Tolerance development to the analgesic effect of tapentadol in the CCI model was twice as slow as that of morphine. It is suggested that the broad analgesic profile of tapentadol and its relative resistance to tolerance development may be due to a dual mode of action consisting of both MOR activation and NE reuptake inhibition.

Rolf terlinden et al [28] proposed Tapentadol is a novel, centrally acting oral analgesic with a dual mode of action that has demonstrated efficacy in preclinical and clinical models of pain relief. The present study investigated and characterized the absorption, metabolism, and excretion of tapentadol in humans. Four healthy male subjects received a single 100-mg oral dose of 3-[^{14}C]-labeled tapentadol HCl for evaluation of the pharmacokinetics of the drug and the excretion balance of radiocarbon. The concentration-time profiles of radiocarbon in whole blood and serum and radiocarbon excretion in the urine and feces, and the expired $C0_2$ were determined. The serum pharmacokinetics and excretion kinetics of tapentadol and its conjugates were assessed, as was its tolerability. Absorption was rapid (with a mean maximum serum concentration [C_{max}], 2.45 µg-eq/m]; a time to C_{max}, 1.25–1.5 h), and the drug was present primarily in the form of conjugated metabolites (conjugated: unconjugated metabolites = 24:1). Excretion of radiocarbon was rapid and complete (>95% within 24 h; 99.9% within 5 days) and almost exclusively renal (99%:69% conjugates; 27% other metabolites; 3% in unchanged form). No severe adverse events or clinically relevant changes in vital signs, laboratory measurements,

electrocardiogram recording, or physical examination findings were reported. In our study group, it was found that a single oral dose of tapentadol was rapidly absorbed, then excreted into the urine, primarily in the form of conjugated metabolites, and was well tolerated.

Thomas M Tzschentke et al(2009) [29] stated that Tapentadol exerts its analgesic effects through micro opioid receptor agonism and nor-adrenaline reuptake inhibition in the central nervous system. Preclinical studies demonstrated that tapentadol is effective in a broad range of pain models, including nociceptive, inflammatory, visceral, mono- and polyneuropathic models. Moreover, clinical studies showed that tapentadol effectively relieves moderate to severe pain in various pain care settings. In addition, it was reported to be associated with significantly fewer treatment discontinuations due to a significantly lower incidence of gastrointestinal-related adverse events compared with equivalent doses of oxycodone. The combination of these reduced treatment discontinuation rates and tapentadol efficacy for the relief of moderate to severe nociceptive and neuropathic pain may offer an improvement in pain therapy by increasing patient compliance with their treatment regimen.

Xu Steven Xu et al(2010**)** [30] **Background** Tapentadol is a new, centrally active analgesic agent with two modes of action - μ opioid receptor agonism and nor-epinephrine reuptake inhibition - and the immediate-release (IR) formulation is approved in the US for the relief of moderate to severe acute pain. The aims of this analysis were to develop a population pharmacokinetic model to facilitate the understanding of the pharmacokinetics of tapentadol IR in healthy subjects and patients following single and multiple dosing, and to identify covariates that might explain variability in exposure following oral administration.

Methods: The analysis included pooled data from 11□385 serum pharmacokinetic samples from 1827 healthy subjects and patients with moderate to severe pain. Population pharmacokinetic

modeling was conducted using nonlinear mixed-effects modeling (NONMEM®) software to estimate population pharmacokinetic parameters and the influence of the subject's demographic characteristics, clinical laboratory chemistry values and disease status on these parameters. Simulations were performed to assess the clinical relevance of the covariate effects on tapentadol exposure.

Results**:** A two-compartment model with zero-order release followed by first-order absorption and first-order elimination best described the pharmacokinetics of tapentadol IR following oral administration. The inter-individual variability (coefficient of variation) in apparent oral clearance (CL/F) and the apparent central volume of distribution after oral administration were 30% and 29%, respectively. An additive error model was used to describe the residual variability in the log-transformed data, and the standard deviation values were 0.308 and 0.314 for intensively and sparsely sampled data, respectively. Covariate analysis showed that sex, age, bodyweight, race, body fat, hepatic function (using total bilirubin and total protein as surrogate markers), health status and creatinine clearance were statistically significant factors influencing the pharmacokinetics of tapentadol. Total bilirubin was a particularly important factor that influenced CL/F, which decreased by more than 60% in subjects with total bilirubin greater than 50□µmol/L.

Conclusions: The population pharmacokinetic model for tapentadol IR identified the relationship between pharmacokinetic parameters and a wide range of covariates. The simulations of tapentadol exposure with identified, statistically significant covariates demonstrated that only hepatic function (as characterized by total bilirubin and total protein) may be considered a clinically relevant factor that warrants dose adjustment. None of the other covariates are of clinical relevance, nor do they necessitate dose adjustment.

Cooper et al [31] developed a sensitive LC-MS/MS method for the quantification of oxycodone, oxymorphone and noroxycodone which has been used to analyze clinical samples. The analytes are well separated on a typical C18, 5 micron HPLC column, but the cycle time of 9 minutes is a hindrance to maximizing throughput. BASi has sought to improve cycle time while also improving resolution by converting the method to utilize an ultra small particle size C18 column with a conventional HPLC.

Methods: The range of the validated assay is 0.1-100 ng for oxycodone and oxymorphone, and 0.5-100 ng/ml for noroxycodone. Detection is by positive Turbo Ion spray on a Sciex API-4000. The HPLC column is a 2.1 X 50 mm, 5 micron, Waters XBridge C18. Typical retention times of the analytes are oxycodone 5.2 minutes, oxymorphone 3.5 minutes, and noroxycodone 2.4 minutes. The cycle time is approximately 9 minutes. For the new, faster method, the chromatography has been altered to accommodate the small particle column [2 x 30 mm, 1.5 micron, Grace Vison HT C18] but the extraction and mass spectrometer conditions remain the same. The analytes are eluted on a steep gradient from 10 to 100% organic with a 1 minute hold at 5 minutes and a flow rate of 0.2 mL/min.

Arunadevi S. Birajdar(2009) [32] developed a high-performance liquid chromatographic method has been developed for the simultaneous analysis of paracetamol and tramadol in combined solid dosage form. The mobile phase consisting of Acetonitrile- 0.26 % Triethylamine buffer (pH 7.3) in ratio of (45:55 % v/v) was delivered at the flow rate of 1.0 mL/min and UV detection was carried out at 264 nm. The separation was achieved using C_{18} reverse-phase column (250 X 4.6 mm I.D., particle size 5µm). The method was linear over the concentration range of 1.0-12.0 µg/mL for Paracetmol and 0.1-1.2 µg/mL for tramadol. Domperidone was used as an internal standard (IS). The analytical recovery obtained was

99.88%. The validation of method carried out as per ICH guidelines. The described HPLC method was successfully employed for the analysis of pharmaceutical formulations containing combined dosage form and can be employed for bioequivalence study in future for the same formulations.

Markus G Gebauer et al(2001) [33] An HPLC method for the quantification of oxycodone and lidocaine in a gel matrix is described. The mobile phase consisted of methanol–water–acetic acid (35:15:1 v/v/v) and was delivered at 1.5 ml/min through a 4.6×250 mm Zorbax® SB-C8 column. Oxycodone was detected at 285 nm and lidocaine at 264 nm. Linear calibration curves were obtained for oxycodone in the range of 0.05–1.5% (w/w) and for lidocaine in the range of 0.1–5.0% (w/w). Oxycodone and lidocaine were treated with hydrogen peroxide and the oxidation products were readily separated on the column. The method was applied to assess the stability of a gel containing oxycodone hydrochloride (0.3% w/w) and lidocaine (1.5% w/w). The gel was stored under refrigeration in ready-to-use syringes and under these conditions oxycodone and lidocaine were stable for at least 1 year. The gel is useful in the management of tenesmus in rectal cancer.

Lambropoulos J et al(1999) [34]The stability indicating properties of the USP method for the assay of fentanyl in fentanyl citrate injection were evaluated [1] by analyzing fentanyl drug substance and product after acid, hydrogen peroxide, heat, and light treatment. N-phenyl-N-(4-piperidinyl) propionamide (PPA), which is a known degradation product/process impurity of fentanyl, was not adequately resolved from the fentanyl peak, and mobile phase adjustments did not improve the resolution (Fig. 1). Therefore, the USP method did not meet the requirements for a stability-indicating assay. In addition, the wavelength in the USP method was too high (230

nm) to provide adequate levels for the quantitation of the related substances of fentanyl and, in addition, the acetate ions in the mobile phase could interfere with a lower wavelength detection. An isocratic, reversed phase, stability indicating, high performance liquid chromatographic (HPLC) method for the assay of fentanyl and related substances in fentanyl citrate injection, USP has been developed and validated. The chromatographic conditions employed an Inertsil C8, 5 column (25 cm x 4.6 mm), a mobile phase of aqueous perchloric acid [0.23%, w/v]-Acetonitrile [65:35, v/v], and ultraviolet (UV) detection at 206 nm. Under the chromatographic conditions of the method, PPA and seven other known process impurities were separated from the active. Degradation studies showed that the active eluted as a spectrally pure peak resolved from its degradation products.

John Lambropoulos et al(2000) [35] A high performance liquid chromatography (HPLC) method for the assay of fentanyl citrate, alfentanil hydrochloride, and sufentanil citrate swab samples was developed and validated in order to control a cleaning procedure. The swabbing procedure involved Super POLX 1200 wipers moistened with water. The assay employed extraction of swabs with water and analysis by isocratic, reversed-phase, HPLC with varying ultraviolet (UV) detection for desired sensitivity, depending on the analyte. The method was shown to be selective and linear from the limits of quantitation (0. 10, 0. 20, and 0.15 µg/swab for fentanyl citrate, alfentanil, and sufentanil, respectively) to over three times these concentrations. The assay limits (detection levels) per swab area were set at least at 0.2% of the concentrations of the actives in the drug products (0.02, 0. 10, and 0. 10 µg/swab or approximately 0.03, 0.02, and 0.2% for fentanyl citrate, alfentanil, and sufentanil, respectively). It should be noted that all active concentrations listed in this work were calculated based on the salt form concentration for fentanyl (citrate salt) and the free base forms for alfentanil and

sufentanil. No reference standard was available for alfentanil hydrochloride and sufentanil citrate. Drug product was used instead throughout this study.

J Lambropoulos et al(1999)[36] proposed the stability indicating properties of the USP method for the assay of fentanyl in fentanyl citrate injection were evaluated [1] by analyzing fentanyl drug substance and product after acid, hydrogen peroxide, heat, and light treatment. N-phenyl-N-(4-piperidinyl) propionamide (PPA), which is a known degradation product/process impurity of fentanyl, was not adequately resolved from the fentanyl peak, and mobile phase adjustments did not improve the resolution (Fig. 1). Therefore, the USP method did not meet the requirements for a stability-indicating assay. In addition, the wavelength in the USP method was too high (230 nm) to provide adequate levels for the quantitation of the related substances of fentanyl and, in addition, the acetate ions in the mobile phase could interfere with a lower wavelength detection. An isocratic, reversed phase, stability indicating, high performance liquid chromatographic (HPLC) method for the assay of fentanyl and related substances in fentanyl citrate injection, USP has been developed and validated. The chromatographic conditions employed an Inertsil C8, 5 column (25 cm x 4.6 mm), a mobile phase of aqueous perchloric acid [0.23%, w/v]-Acetonitrile [65:35, v/v], and ultraviolet (UV) detection at 206 nm. Under the chromatographic conditions of the method, PPA and seven other known process impurities were separated from the active. Degradation studies showed that the active eluted as a spectrally pure peak resolved from its degradation products.

Amir mehdizadeh et al(2005) [37] developed a simple, sensitive and specific HPLC method and also a simple and fast extraction procedure were developed for quantitative analysis of fentanyl transdermal patches. Chloroform, methanol and ethanol were used as extracting solvents with recovery percent of 92.1, 94.3 and 99.4% respectively. Fentanyl was extracted with

ethanol and the eluted fentanyl through the C18 column was monitored by UV detection at 230 nm. The linearity was at the range of 0.5-10 µg/mL with correlation coefficient (r2) of 0.9992. Both intra and inter-day accuracy and precision were within acceptable limits. The detection limit (DL) and quantitation limit (QL) were 0.15 and 0.5 µg/mL, respectively. Other validation characteristics such as selectivity, robustness and ruggedness were evaluated. Following method validation, a system suitability test (SST) including capacity factor (k´), plate number (N), tailing factor (T), and RSD was defined for routine test.

Finally after searching of the all literature no method was developed for estimation of Tapentadol Hydrochloride by RP-HPLC. So by this, I have decided to do method development on this drug by RP-HPLC.

DRUG PROFILE

TAPENTADOL HYDROCHLORIDE

5.1 Chemical profile [38, 39]

Brand name:

Nucynta, Nucynta ER, Niap

Chemical name/IUPAC NAME:

3-[(1R, 2R)-3-(Dimethylamine)-1-ethyl-2-methylpropyl] phenol hydrochloride

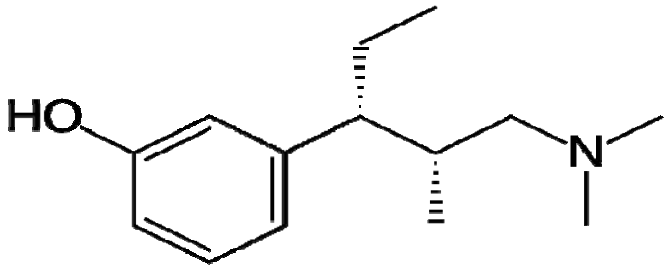
Molecular formula:

$C_{14}H_{23}NO$ .HCL

Mol. mass: 221.339 g/mo

Structure:

Fig: 2 MOLECULAR STRUCTURE OF TAPENTADOL HYDROCHLORIDE



.HCL

Pharmacokinetic data:

Bioavailability : 31.9 ± 6.8% (oral)

Half life : 4 hrs 30 to 50hours.

Metabolism : Hepatic glucuronidation and sulfate conjugation

Half-life : 4 hrs

Excretion : Renal (>95%) and feca

Routes : Oral, Oral, Other ROA Unknown

Description:

White or off white powder

Category [40]:

Centrally acting analgesic, agonist at the m-opioid receptor,

Dose [41]:

Immediate release:

50 mg, 75 mg, or 100 mg orally every 4 to 6

Extended release:

Opioid naive: Initial: 50 mg twice daily (recommended interval: 12 hours); titrate in increments of 50 mg no more frequently than twice daily every 3 days to effective dose (therapeutic range: 100 to 250 mg twice daily) (maximum dose: 500 mg/day)

5.2 Pharmacological profile [42]

Mechanism of action:

Tapentadol is a centrally-acting synthetic analgesic. Although its exact mechanism is unknown, analgesic efficacy is thought to be due to mu-opioid agonist activity and the inhibition of nor-epinephrine reuptake.

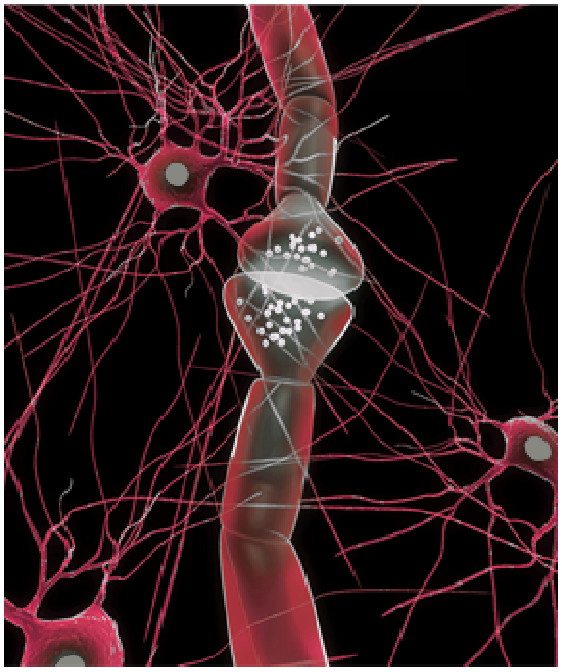


Fig: 3 M.O.A of Tapentadol Hydrochloride

Pharmacodynamics

Tapentadol is a centrally-acting synthetic analgesic. It is 18 times less potent than morphine in binding to the human mu-opioid receptor and is 2–3 times less potent in producing analgesia in animal models. Tapentadol has been shown to inhibit nor-epinephrine reuptake in the brains of rats resulting in increased nor-epinephrine concentrations. In preclinical models, the analgesic activity due to the mu-opioid receptor agonist activity of tapentadol can be antagonized by selective mu-opioid antagonists (e.g., naloxone), whereas the nor-epinephrine reuptake inhibition is sensitive to nor-epinephrine modulators. Tapentadol exerts its analgesic effects without a pharmacologically active metabolite.

Effects on the cardiovascular system: There was no effect of therapeutic and supra therapeutic doses of tapentadol on the QT interval. In a randomized, double-blind, placebo- and positive-

controlled crossover study, healthy subjects were administered five consecutive doses of Nucynta® 100 mg every 6 hours, Nucynta® 150 mg every 6 hours, placebo and a single oral dose of moxifloxacin. Similarly, Nucynta® had no relevant effect on other ECG parameters (heart rate, PR interval, QRS duration, and T-wave or U-wave morphology).

Pharmacokinetics

Absorption

Mean absolute bioavailability after single-dose administration (fasting) is approximately 32% due to extensive first-pass metabolism. Maximum serum concentrations of tapentadol are typically observed at around 1.25 hours after dosing.

Dose-proportional increases in the Cmax and AUC values of tapentadol have been observed over the 50 to 150 mg dose range.

A multiple (every 6 hour) dose study with doses ranging from 75 to 175 mg tapentadol showed a mean accumulation factor of 1.6 for the parent drug and 1.8 for the major metabolite tapentadol-O-glucuronide, which are primarily determined by the dosing interval and apparent half-life of tapentadol and its metabolite.

Food Effect

The AUC and Cmax increased by 25% and 16%, respectively, when Nucynta® was administered after a high-fat, high-calorie breakfast. Nucynta® may be given with or without food.

Distribution

Tapentadol is widely distributed throughout the body. Following intravenous administration, the volume of distribution (Vz) for tapentadol is 540 +/- 98 L. The plasma protein binding is low and amounts to approximately 20%.

Metabolism and Elimination

In humans, the metabolism of Tapentadol Hydrochloride is extensive. About 97% of the parent compound is metabolized. Tapentadol is mainly metabolized via Phase 2 pathways, and only a small amount is metabolized by Phase 1 oxidative pathways. The major pathway of tapentadol metabolism is conjugation with glucuronic acid to produce glucuronide. After oral administration approximately 70% (55% O-glucuronide and 15% sulfate of tapentadol) of the dose is excreted in urine in the conjugated form. A total of 3% of drug was excreted in urine as unchanged drug. Tapentadol is additionally metabolized to N-desmethyl tapentadol (13%) by CYP2C9 and CYP2C19 and to hydroxyl tapentadol (2%) by CYP2D6, which are further metabolized by conjugation. Therefore, drug metabolism mediated by cytochrome P450 system is of less importance than phase 2 conjugation.

None of the metabolites contributes to the analgesic activity.

Tapentadol and its metabolites are excreted almost exclusively (99%) via the kidneys. The terminal half-life is on average 4 hours after oral administration. The total clearance is 1530 +/- 177 ml/min.

Special Populations

Elderly

The mean exposure (AUC) to tapentadol was similar in elderly subjects compared to young adults, with a 16% lower mean Cmax observed in the elderly subject group compared to young adult subjects.

Renal Impairment

AUC and Cmax of tapentadol were comparable in subjects with varying degrees of renal function (from normal to severely impaired). In contrast, increasing exposure (AUC) to tapentadol-O-glucuronide was observed with increasing degree of renal impairment. In subjects with mild, moderate, and severe renal impairment, the AUC of tapentadol-O-glucuronide are 1.5-, 2.5-, and 5.5-fold higher compared with normal renal function, respectively.

Hepatic Impairment

Administration of Nucynta® resulted in higher exposures and serum levels to tapentadol in subjects with impaired hepatic function compared to subjects with normal hepatic function. The ratio of tapentadol pharmacokinetic parameters for the mild and moderate hepatic impairment groups in comparison to the normal hepatic function group were 1.7 and 4.2, respectively, for AUC; 1.4 and 2.5, respectively, for Cmax; and 1.2 and 1.4, respectively, for $t_{1/2}$. The rate of formation of tapentadol-O-glucuronide was lower in subjects with increased liver impairment.

Pharmacokinetic Drug Interactions

Tapentadol is mainly metabolized by Phase 2 glucuronidation, a high capacity/low affinity system; therefore, clinically relevant interactions caused by Phase 2 metabolism are unlikely to occur. Naproxen and probenecid increased the AUC of tapentadol by 17% and 57%,

respectively. These changes are not considered clinically relevant and no change in dose is required.

No changes in the pharmacokinetic parameters of tapentadol were observed when acetaminophen and acetylsalicylic acid were given concomitantly.

In vitro studies did not reveal any potential of tapentadol to either inhibit or induce cytochrome P450 enzymes. Thus, clinically relevant interactions mediated by the cytochrome P450 system are unlikely to occur.

The pharmacokinetics of tapentadol were not affected when gastric pH or gastrointestinal motility were increased by omeprazole and metoclopramide, respectively.

Plasma protein binding of tapentadol is low (approximately 20%). Therefore, the likelihood of pharmacokinetic drug-drug interactions by displacement from the protein binding site is low.

AIM AND OBJECTIVE OF THE STUDY

Analytical chemistry may be defined as the science and art of determining the composition of materials of the elements or compounds contained.

Importance of analytical chemistry is to gain information about the qualitative and quantitative composition of substance and chemical species, that is, to find out what a substance is composed of and exactly how much it is present.

Drug analysis plays an important role in the development of drugs, their manufacture and therapeutic use. Pharmaceutical industries rely up on quantitative chemical analysis to ensure that the raw material used and the final product obtained meets the required specification. For the estimation of drug present in dosage forms, HPLC, HPTLC, and UV-Spectroscopic methods are considered to be the most suitable. These methods are powerful, extremely precise, accurate, sensitive, specific, linear and rapid in analyzing the sample. The chromatographic methods was found to be most suitable than the UV-Spectroscopic due to its very high sensitivity.

These methods are powerful, extremely precise, accurate, sensitive, specific, linear and rapid in analyzing the sample.

Tapentadol Hydrochloride is a centrally acting analgesic, agonist at the mu-opioid receptor activity and the inhibition of nor-epinephrine reuptake.

From the extensive literature review it was found that no method have been reported for estimation of Tapentadol Hydrochloride by using RP-HPLC method,

The present study is planned to develop an analytical method for the determination of Tapentadol Hydrochloride in tablet dosage form by RP-HPLC method under different chromatographic conditions.

The reasons for developing RP-HPLC method for determination of this drug in tablet dosage form is as follows:

1. To develop new RP-HPLC Method by Isocratic Mode.
2. To develop a less run time Method.
3. To carry out estimation of Tapentadol Hydrochloride by using different chromatographic conditions.

Advantages of less run time in HPLC:

- It's beneficial to the company economically.
- To estimate the different compounds with less time in different formulations like tablets, capsules, syrups, expectorants and injections.
- Utilisation of minimum solvent.
- Reduce the cost.
- Less utilisation of men, machine and materials.

PLAN OF WORK

The work plan was to develop Method for Tapentadol Hydrochloride and the plan of present work is as follows:

HPLC:

The work was plan to divide into **three phases**

Phase I:

Optimization of chromatographic conditions

- Selection of wavelength
- Selection of initial separation conditions
- Selection of mobile phase (pH, peak modifier, solvent strength, ratio and flow rate)
- Nature of the stationary phase
- Selection of internal standard
- Selection of separation method and agent

Phase II:

Validation of the method

The developed method were proposed to be validated using the various validation parameters such as,

- Accuracy
- Precision
- Linearity and of detection (LOD) / Limit of quantitation (LOQ)

- Selectivity / Specify
- System suitability.

Phase III:

Estimation of Tapentadol Hydrochloride in a branded tablet dosage form **"NIAP".**

METHODOLOGY

RP-HPLC Method Development and Validation for the Estimation of Tapentadol Hydrochloride in Marketed Tablet dosage form

MATERIALS AND ISTRUMENTS USED

a) Drug sample & Study products

Tapentadol Hydrochloride was obtained from MSN Laboratories Ltd, Hyderabad, India.

Test product:

Tapentadol Hydrochloride (niap) (Reddy's Laboratories Ltd) Tablets were purchased from the local market.

b) Chemicals and solvents used for estimation:

- HPLC Water – Qualigens, Mumbai, India.
- Acetonitrile – Rankem, Mumbai, India.
- Distilled water – Double Distilled water.
- Potassium dihydrogen phosphate – M.R.Fine chem, Banglore.
- NaOH Base – Manas chemicals, Mumbai.

c) Instruments used:

- Elico pH meter LI 127.
- Water 2695 AT HPLC.
- Photo diode array detector (waters 2996)
- Shimadzu 1700 UV Spectrophotometer.

- Sonica Ultrasonic cleaner.
- Solvent filtration unit – Millipore.
- Shimadzu electronic balance AX 200.
- Ultra Cooling centrifuge – Remi, India.

OPTIMIZATION OF CHROMATOGRAPHIC CONDITION FOR THE ESTIMATION OF TAPETADOL HYDROCHLORIDE

Selection of wavelength

An UV spectrum of 10 µg/ml Tapentadol Hydrochloride in water was recorded by scanning in the range of 200 nm to 400 nm. From the UV spectrum wavelength of 215 nm was selected. At this wavelength Tapentadol Hydrochloride showed good absorbance.

Selection of chromatographic method

Proper selection of the method depends upon the nature of the sample (ionic/ionisable/neutral molecule), its molecular weight and solubility. The drug selected in the present study is polar in nature and hence the reverse phase HPLC was selected for the initial separations because of its simplicity and suitability.

Initial separation conditions

Standard solution: 10 µg/ml of Tapentadol Hydrochloride in HPLC grade water.

Equipment

System : Water 2695 AT HPLC.

Pump : waters 2695 prominence solvent delivery system

Detector : Photo diode array detector (waters 2998)

Injector : Auto-sampler

Chromatographic conditions

Stationary phase : Phenomenex C_{18} Column

Mobile phase : Solvent A: 0.5% glacial acetic acid

Solvent B: Acetonitrile

Solvent ratio : 50:50

Detection : 215 nm

Flow rate : 1.0 ml/min

Sample size : 20 µl

Needle wash : water HPLC grade

Column temperature : room temperature (20ºC)

Chromatographic condition 1

Stationary phase : Phenomenex C18 Column

Mobile phase : Solvent A: 0.5% Glacial acetic acid (pH- 3.5)

Solvent B: Acetonitrile + Methanol (50+50)

Solvent ratio : 60:40(A: B)

Detection : 215 nm

Flow rate : 1.0 ml/min

Chromatographic condition 2

Stationary phase	: Phenomenex C18 Column
Mobile phase	: Solvent A: ortho phosphoric acid (pH- 3.5)
	Solvent B: Acetonitrile + Methanol (50+50)
Solvent ratio	: 70:30(A: B)
Detection	: 215 nm
Flow rate	: 0.9 ml/min

Chromatographic condition 3

Stationary phase	: Phenomenex C18 Column
Mobile phase	: Solvent A: Water
	Solvent B: Acetonitrile + Methanol (50+50)
Solvent ratio	: 70:30(A: B)
Detection	: 215nm
Flow rate	: 1.0 ml/min

Chromatographic condition 4

Stationary phase	: Phenomenex C18 Column
Mobile phase	: Solvent A: Water
	Solvent B: Acetonitrile
Solvent ratio	: 75:25(A: B)

Detection : 215 nm

Flow rate : 1.3 ml/min

Chromatographic condition 5

Stationary phase : Phenomenex C18 Column

Mobile phase : Solvent A: Water

Solvent B: Methanol

Solvent ratio : 75:25(A: B)

Detection : 215nm

Flow rate : 1.0 ml/min

Effect of ratio of mobile phase

Phosphate Buffer and Methanol with 50:50, 70:30, 40:60 and 85:15 ratios were used as the mobile phase. At 50:50 ratio, the symmetric peak was eluted at 4.26 for Tapentadol Hydrochloride. At 70:30 and 75:25 ratios the peaks were asymmetric. Thus for the present study 50:50 ratio mobile phase system was selected.

Effect of flow rate

Flow rate of 1.0 to 1.5 ml/min were used and chromatograms were recorded. When 1.23 ml/min was used, elution time of peak was 4.26. This flow rate gave symmetric and well retained peak. So for the present study, 1.23 ml/min was selected.

Finally, the following Fixed Chromatographic Conditions were selected for the estimation of Tapentadol Hydrochloride.

Stationary phase	: Phenomenex C18 Column
Mobile phase	: Solvent A: Phosphate Buffer adjusted to Ph 7 With NaOH, Solvent B: Acetonitrile
Solvent ratio	: 50:50(A: B)
Detection	: 215 nm
Flow rate	: 1.23 ml/min
Sample size	: 20 µl
Needle wash	: Water HPLC grade
Column temperature	: Room temperature of above 20^0C

QUANTIFICATION AND VALIDATION

Preparation of Standard Stock Solution

Standard stock solution of 1000 µg/ml of Tapentadol Hydrochloride was prepared using HPLC grade water. Further, 50µg/ml solution was prepared from the stock solution by appropriate dilution with mobile phase. Working standard solution was prepared by diluting the 0.5, 0.75, 1.0, 1.25, and 1.50ml of standard solution with 10 ml of mobile phase to give the concentration of 50-150 µg/ml. All the solutions are filtered through 0.45µ GHP filter.

Preparation of Sample Solution

Weigh accurately 6 tablets of Tapentadol Hydrochloride. Take powder equivalent to 50mg in a 50 ml volumetric flask. Dissolve it with water, sonicate for 10min and make up the volume with the same solvent. From this above stock solution pipette out 1ml to10ml volumetric flask and make up the volume with mobile phase containing 50:50 ratio and filter through 0.45µ GHP filter.

Recording of chromatogram

After optimization of chromatographic conditions mentioned above, a steady baseline for about 8min was recorded. After the stabilization of the baseline, 100 µg/ml of the standard solution was injected and chromatogram was recorded until the reproducibility of the peak areas was found satisfactory. The standard solutions containing 50-150 µg/ml of Tapentadol Hydrochloride was injected and chromatograms were recorded.

Retention time of Tapentadol Hydrochloride was found to be 4.23 and shown in **Fig: 8, 9, 10, 11 and 12** respectively.

The procedure was repeated using for the both sample and standard solutions and the chromatogram was shown in **Fig: 7, 13.**

Calibration curves were plotted using peak area against concentration of corresponding standard solutions. Peak areas of the sample chromatograms were reordered and the amount of Tapentadol Hydrochloride was calculated from the regression equation.

VALIDATION OF THE HPLC METHOD

Validation is a process of establishing documented evidence, which provides a high degree of assurance that a specific activity will consistently produce a desired result or product meeting its predetermined specifications and quality characteristics**.**

Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, quality, purity, and potency of the drug substances and drug products. Simply, method validation is the process of proving that an analytical method is acceptable for its intended purpose. A successful validation guarantees that both the technical and regulatory objectives of the analytical methods have been fulfilled. The transfer of a method is best accomplished by a systematic method validation process. The real goal of validation process is to challenge the method and determine limits of allowed variability for the conditions needed to run the method.

Significance of Method Validation:

The quality of analytical data is a key factor in the success of a drug development program. The process of method development and validation has a direct impact on the quality of these data.

- To trust the method.
- Regulatory requirement.

Analytical validation is a very important feature of any package of information submitted to international regulatory agencies in support of new product marketing or clinical trials applications. A thorough method development can almost rule out all potential problems, at the same time, a thorough validation programmed can address the most common ones and provide assurance to the intended purpose (can be used with 100% confidence). In other words, a thorough validation can fulfill all the technical and regulatory objectives. A direct consequence and most significant out come from any method validation exercise is 'the development of meaningful specifications can be predicted upon the use of validated analytical procedures that can assess changes in a drug substance or drug product during its life time.

Analytical characteristics listed below may not be applicable to every test procedure or every particular material. It will mostly depend on the purpose for which the procedure is required, however, these following aspects of validation should be given due importance.

VALIDATION OF THE METHOD

The development of HPLC method for the estimation of Tapentadol Hydrochloride in a dosage form, validation of the method was performed. The validation procedure for the developed method is as follows.

a) Accuracy:

Accuracy of the method was determined by recovery experiments. The reference standards of the respective drug were added to the sample solution at the level of 50% (6), 100% (3) and 150% (6). These were further diluted by procedure as followed in the estimation of formulation. The concentrations of the drugs present in the resulting sample solution were determined by using assay method.

b) Precision:

Repeatability and reproducibility studies were carried out for the precision of the method. Repeatability studies were done by consequently injecting the standard solution of same concentration i.e., 100 µg/ml of Tapentadol Hydrochloride (n=6). These solutions were prepared in duplicate and injected as per assay procedure.

c) Linearity and range:

From the standard stock solutions, a series of standard solution was prepared to contain 50µg/ml to 150µg/ml. The solutions were examined by the assay procedure. The calibration curve was plotted using peak area Vs concentration of the standard solution. From the calibration curve, the slope and intercept were calculated.

d) Limit of detection (LOD) and Limit of quantification (LOQ):

The LOD and LOQ were separately determined and reported in **table no: 9**, based on the calibration curve of standard solution. The residual standard deviation of the regression line or the standard deviation of y – intercepts of regression lines may be used to calculate LOD and LOQ. LOD = 3.3 × D/S and LOQ = 10 × D/S, where, D is the Standard deviation of y – intercepts of regression line and S is the slope of the calibration curve.

e) System Suitability Studies:

The system suitability studies were carried out as specified in USP. These parameters include column efficiency, resolution and capacity factor.

g) Ruggedness:

The sample was analyzed by a different chemist in the same instruments on a different day and the results are as follows:

1) First Chemist:

For tablet:

Tapentadol Hydrochloride: 50 mg / average weight of the tablet

2) Second chemist:

For tablet:

Tapentadol Hydrochloride: 50 mg / average weight of the tablet

The deviation between the results of the two chemists is 0.47% for Tapentadol Hydrochloride. Limit was not more than 2.0%.

The system suitability was also found to be within the limits.

The deviation among the results obtained by two chemists on a different day is well within the limits. Hence the method is rugged.

Capacity factor (k') is a measured of how well the sample molecule is retained by a column during an isocratic separation .The ideal value of (k') ranges from 2-10.

Capacity Factor (k') = $V_1 – V_0 / V_0$

Where, V_1 is the retention volume at the apex of the peak (solute) and V_0 is the void volume of the system.

Resolution (Rs) is the difference between the retention times of two solutes divided by their average peak width. The ideal value of (Rs) is 1.5

Resolution (Rs) = $Rt_1 - Rt_2 / 0.5(W_1 - W_2)$

Where, Rt_1 and Rt_2 are the retention times of component 1 and 2, respectively.

Selectivity (α) is a measured of relative retention of two components in a mixture. The ideal value of α is 2

Selectivity (α) = $V_2 - V_0 / V_1 - V_0$

Where, V_0 is the void volume of column and V_2 and V_1 are the retention volume of the second and first peaks, respectively.

Efficiency (N) of a column is measured by the number of theoretical plates per meter .Column with N ranging from 5,000 to 100,000 plates/meters are ideal for good separation.

Column efficiency (N) = R_t^2 / W^2

Where R_t is the retention time and W is the peak width.

Peak asymmetry factor can be used as a criterion of column performance. For a well packed column, an asymmetry factor of 0.9 to 1.1 should be achievable.

Peak asymmetry factor (As) = b / a

Where a and b are the distances on either side of the peak midpoint.

RESULTS AND DISCUSSION

HPLC METHOD

A RP-HPLC method was developed for the estimation of Tapentadol Hydrochloride in tablet dosage form, which can be conveniently employed for routine quality control in pharmaceutical dosage forms. The peak area of standard and sample solutions was calculated. The assay procedures were repeated for 6 times and mean peak area, mean weight of standard drugs, mean weights of sample taken for assay were calculated.

The percentage of drug found in formulation, mean and relative standard deviation were calculated and presented in **Table 4.** The result of analysis shows that the amount of drug present in the formulation has a very good correlation with the label claim of the formulation.

Validation of the method

The accuracy of the method was determined by recovery experiments. A known quantity of the pure drug was added to the pre-analyzed sample formulations at 50%, 100% and 150% levels. The recovery studies were carried out 6 times of each level and the percentage recovery and percentage relative standard deviation were calculated and given in **Table 5.**

The percentage recovery of Tapentadol Hydrochloride was found to be in the range of 99.6% and 100.26% respectively from the data obtained, it was observed that the recoveries of standard drugs were found to be accurate and within the specified limits.

Table no: 4 Analysis of Formulations

Drug	Label Claim (mg/tablet)	Estimated Amount (mg/tablet)	% Label Claim	%RSD*
Tablet	50.0	49.93	99.88	0.3944

*each value is a mean of six observations

The precision of the method was determined by inter day and intraday studies. The area of drug peak and percentage relative standard deviation of intraday and inter day were calculated and presented in **Table 6(a)** and **6(b)** respectively. The results revealed that the developed method was found to be reproducible in nature.

1. Accuracy, precision and recovery:

Table no 5: Accuracy (Recovery studies)

Drug	Label Claim mg/tab	Spike Level (%)	Amount of drug added (mg)	Amount of drug recovered (mg)	Mean Peak area	Percentage Recovery	%RSD*
Tapentadol Hydrochloride	50	50	50	49.56	80253.6	100.26	0.57
		100	100	99.43	159405.5	101.4	0.56
		150	150	150.24	236914	99.6	0.63

*-Each value is a mean of six observations.

Acceptance Criteria:

For an assay method, mean recovery will be 100% ± 2% at each concentration over the range of 50-150% of the target concentration. From the data obtained, it was observed that the recoveries of standard drugs were found to be accurate and within the specified limits.

2. Precision

Table 6(a): Intraday Studies

No of Injection	Conc. of Tapentadol Hydrochloride (µg/ml)	Peak Area	% RSD
6	100	159490 158750 159324 159545 158905 159410	0.9

*-Each value is a mean of six observations.

Acceptance criteria

The % relative standard deviation of peak areas of Tapentadol Hydrochloride should not be more than 2.0

Table no 6b: Inter day studies

Day	Conc. of Tapentadol Hydrochloride (µg/ml)	Peak Area	% RSD
0Day 1	50	80270 80256 80235 80545 80385 81395	0.83
Day 2	100	159540 159425 159485 159360 159365 159580	0.65
Day 3	150	237982 240195 233165 239423 236540 240882	0.71

Acceptance criteria

The % relative standard deviation of peak areas of Tapentadol Hydrochloride should not be more than 2.0

3. **Linearity:**

Tapentadol Hydrochloride was found to be linear in the range of 50 to 150µg/ml and shown in the table no: 7. The correlation coefficient of Tapentadol Hydrochloride was found to be 0.999.The calibration curves were plotted as peak area Vs concentration of the standard solutions (**Fig: 4)**.

The calibration graph shows that linear response was obtained over the range of concentrations used in the assay procedure. The range demonstrates that the method is linear outside the limits of expected use.

Table no: 7 Linearity range of Tapentadol Hydrochloride

S.NO.	Conc. of Tapentadol Hydrochloride (µg/ml)	Peak area of Tapentadol Hydrochloride
1	50	80252
2	75	121487
3	100	159408
4	125	197643
5	150	236917

Fig 17: Calibration curve of Tapentadol Hydrochloride

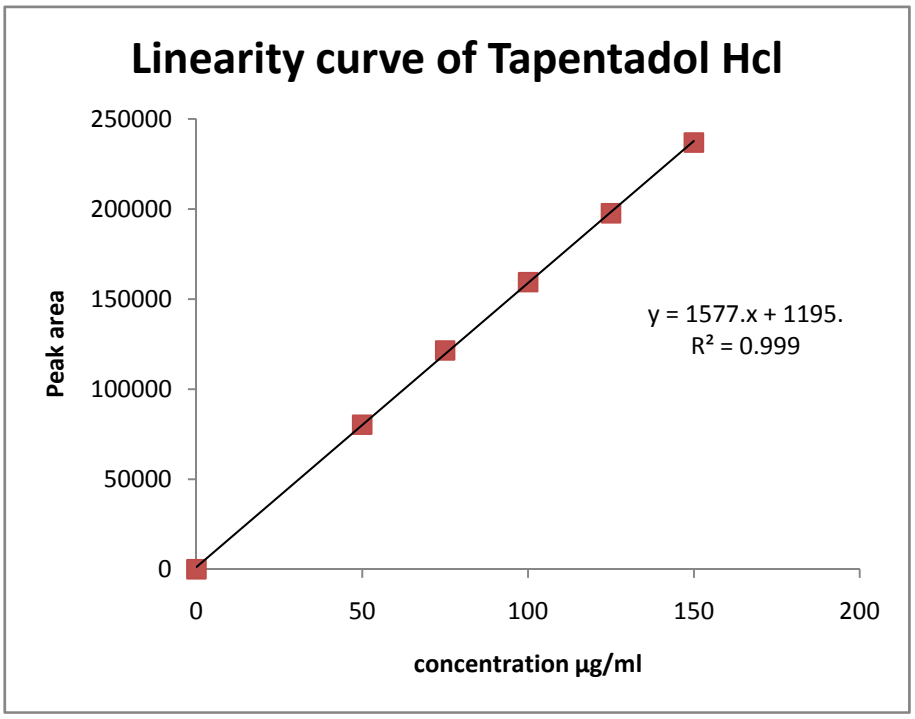


Fig: 4 Linearity curve of Tapentadol Hydrochloride

Acceptance Criteria

Coefficient of correlation should be not less than 0.999

4. Ruggedness:

Table no 8: Ruggedness studies correction

Drug name	**Concentration (µg/ml)**	**Mean peak area**	**%RSD**
Day-1 analyst-1			
Tapentadol Hydrochloride	50	80252	0.615
Day-2 analyst-2			
Tapentadol Hydrochloride	50	83048	0.613

The sample was analyzed by a different chemist and same instruments on a different day have been performed.

The deviation among the results obtained by two chemists on a different day is well within the limits. Hence the method is rugged.

5. Limit of Detection and Limit of Quantification:

The LOD is the smallest concentration of the analyte that gives a measurable response (signal to noise ratio of 3.3). LOD of Tapentadol Hydrochloride was found to be128.88. The LOQ is the smallest concentration of the analyte, which gives response that can be accurately quantified (signal to noise ratio of 10). The LOQ of Tapentadol Hydrochloride was found to be 390.54.

6. System Suitability: correction

The system suitability studies were performed for the standard solution and were presented in **Table no: 9.** the values obtained demonstrated the suitability of the system for the analysis of the above drug combination.

Table no: 9 System suitability studies

Validation Parameters	Tapentadol Hydrochloride
Linearity range (µg/ml)	50-150
Correlation co-efficient (r^2)	0.999
LOD (µg/ml)	128.88
LOQ (µg/ml)	390.54
Intraday (%RSD)*	0.9
Interday (%RSD)*	0.73
Accuracy (%)	99.6-100.26
Resolution factor (Rs)	1.38
No. of theoretical plates (N)	2032.49
Capacity factor (K')	0.36
High equivalent to theoretical plates (HETP)	32.69
Tailing factor	0.68

*-Each value is a mean of six observations.

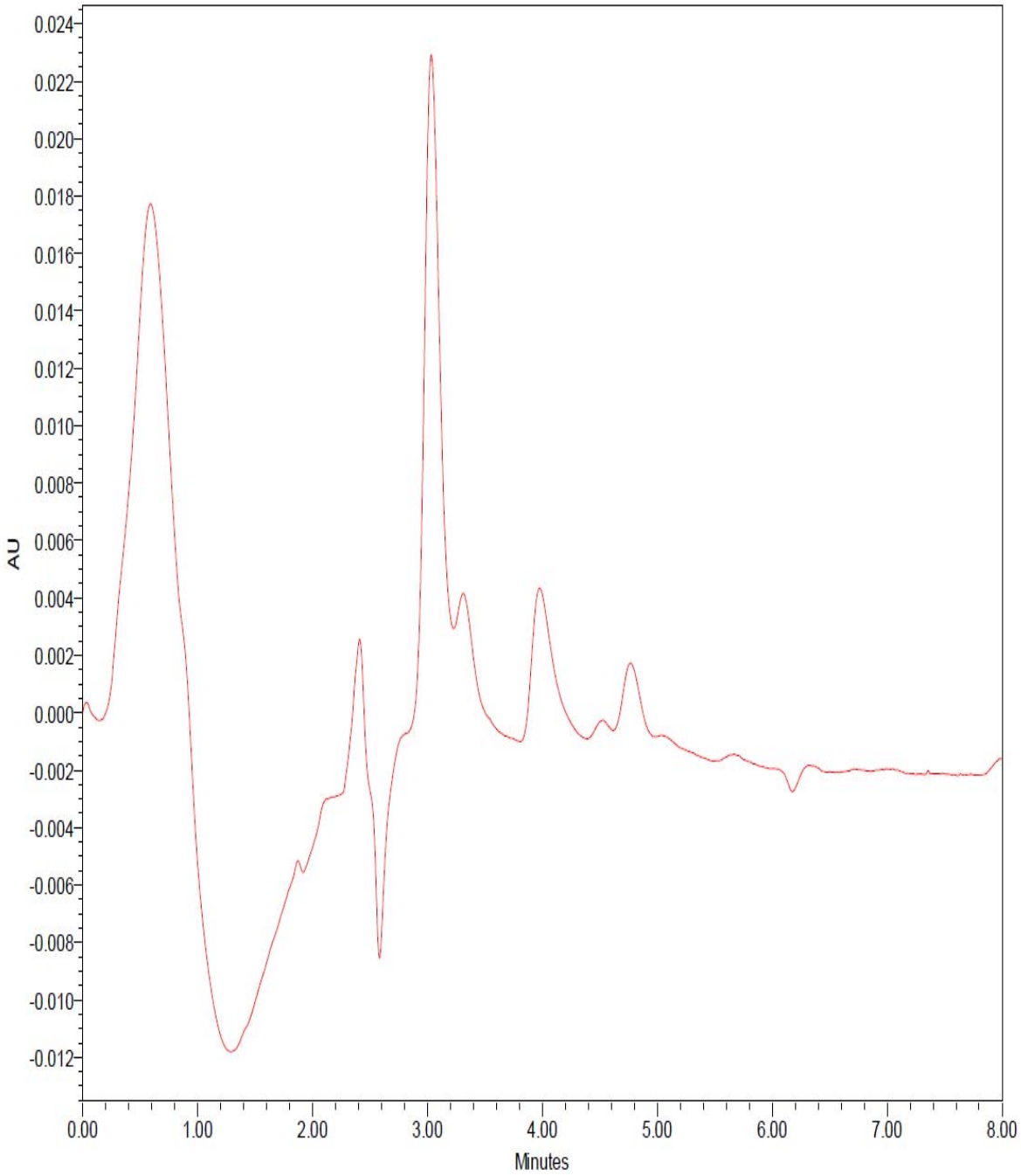


Fig: 5 Chromatogram of Blank without Standard/drug

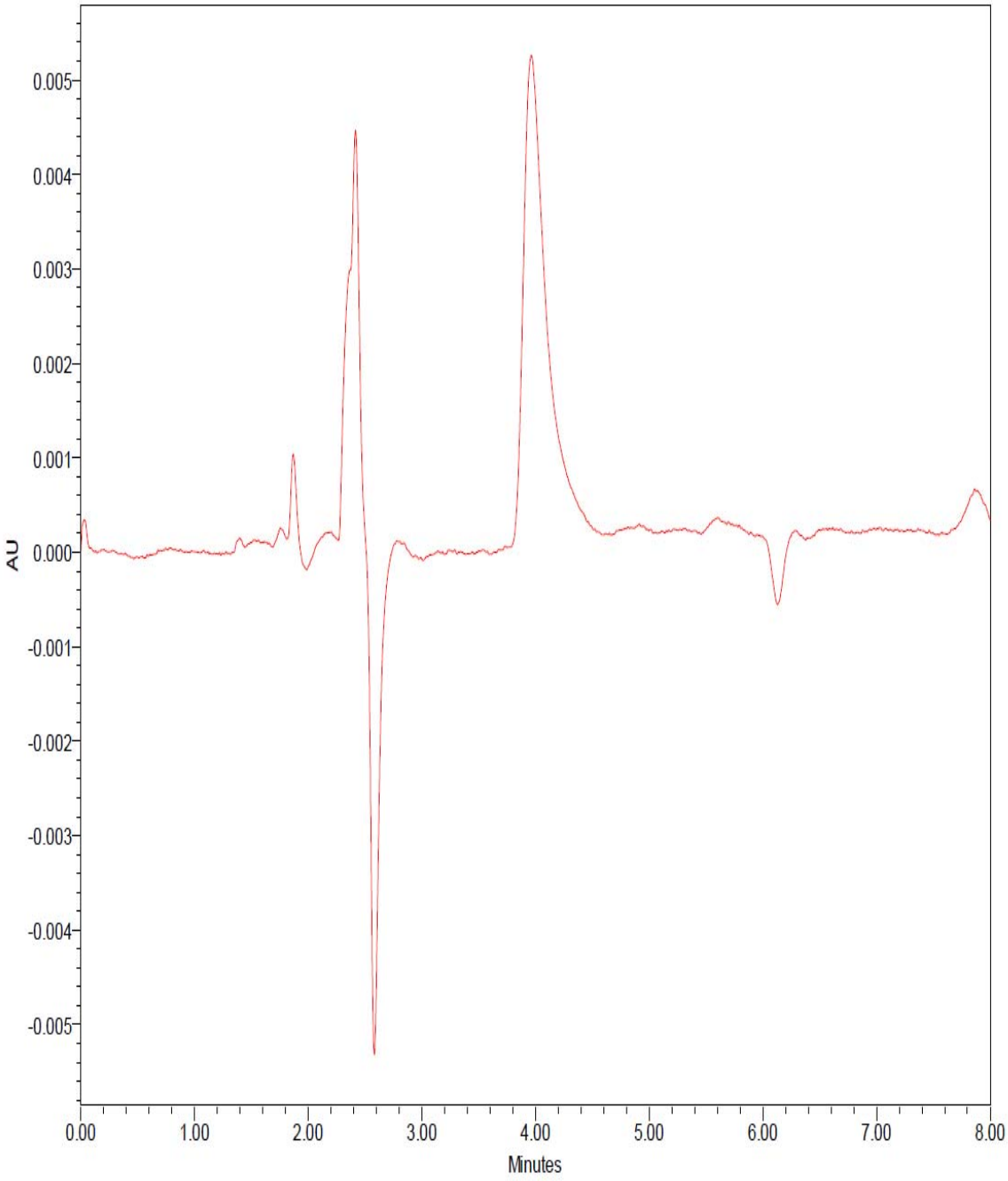


Fig: 6 Chromatogram of Placebo

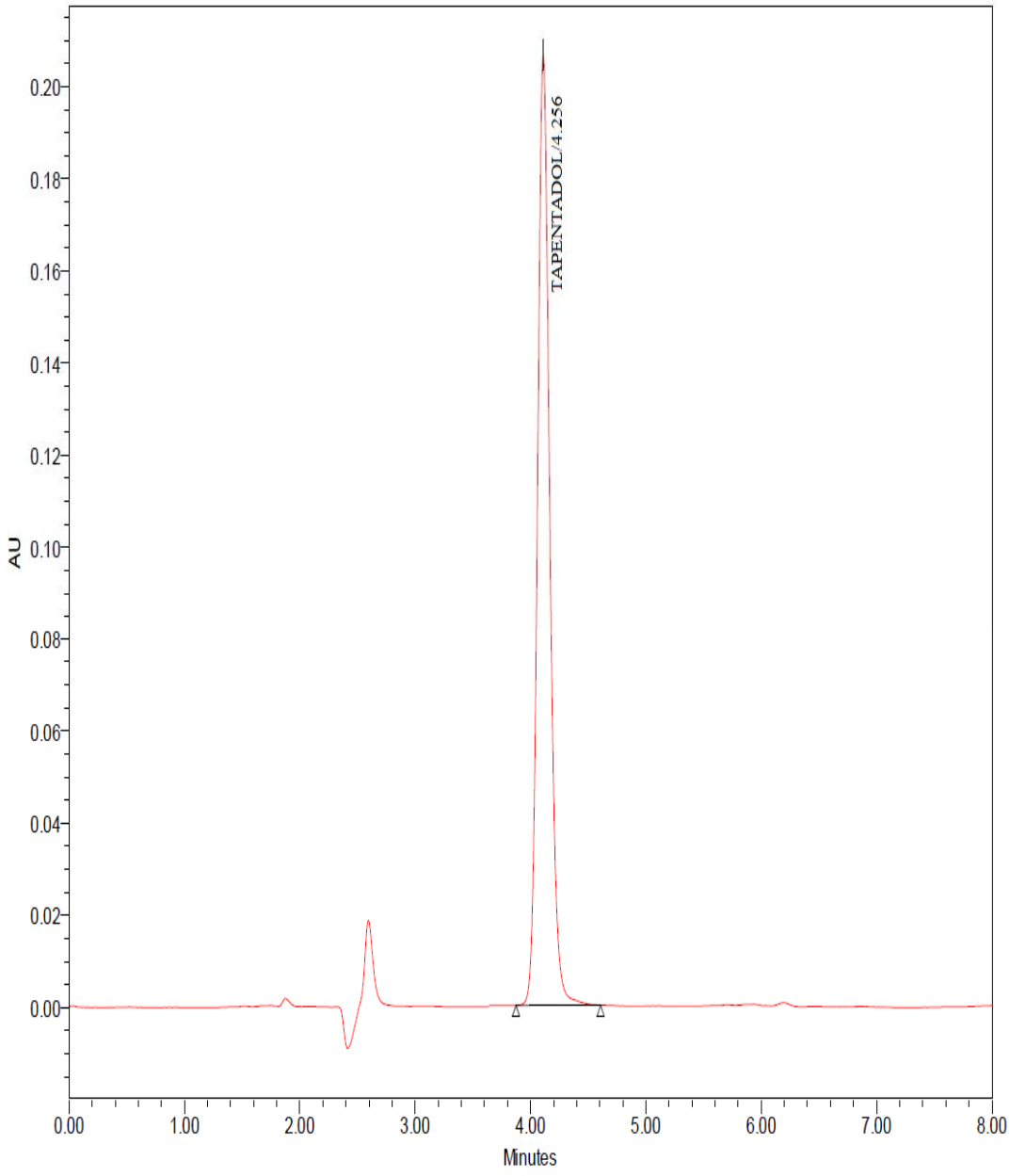


Fig: 7 Standard chromatogram of Tapentadol Hydrochloride (100ug/ml)

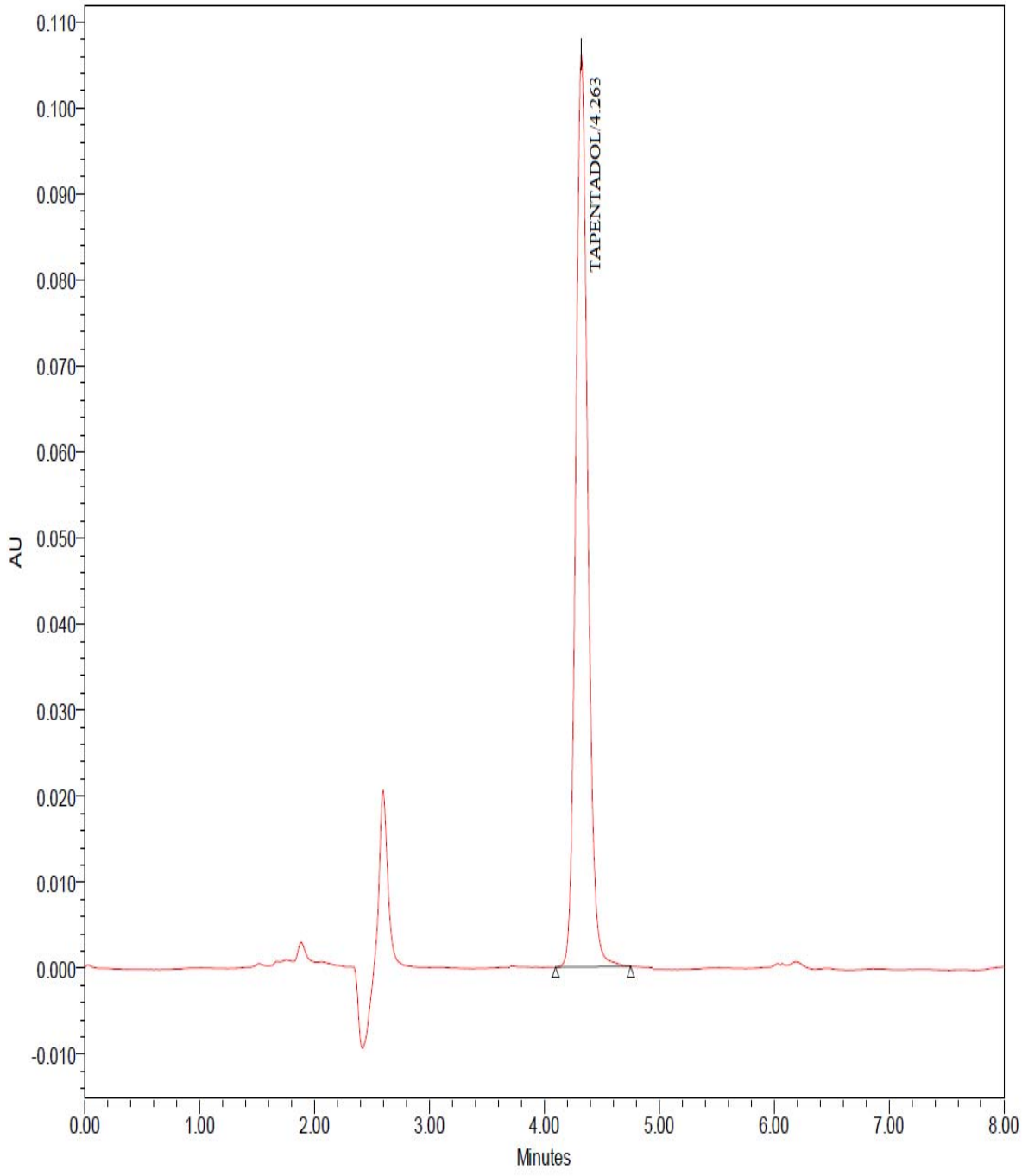


Fig: 8 Chromatogram of Linearity Standard solution of Tapentadol Hydrochloride (50ug/ml)

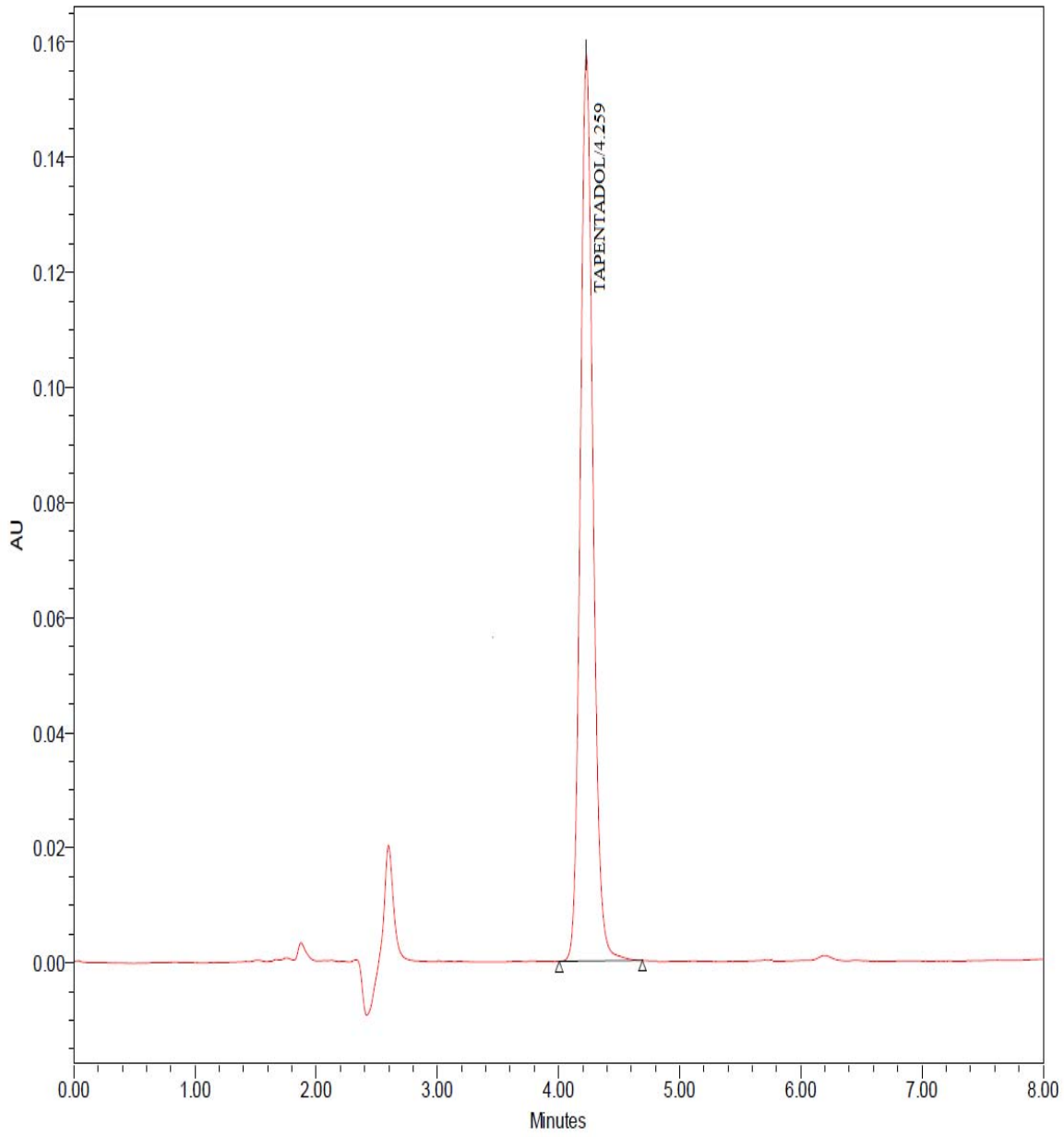


Fig: 9 Chromatogram of Linearity Standard solution of Tapentadol Hydrochloride (75ug/ml)

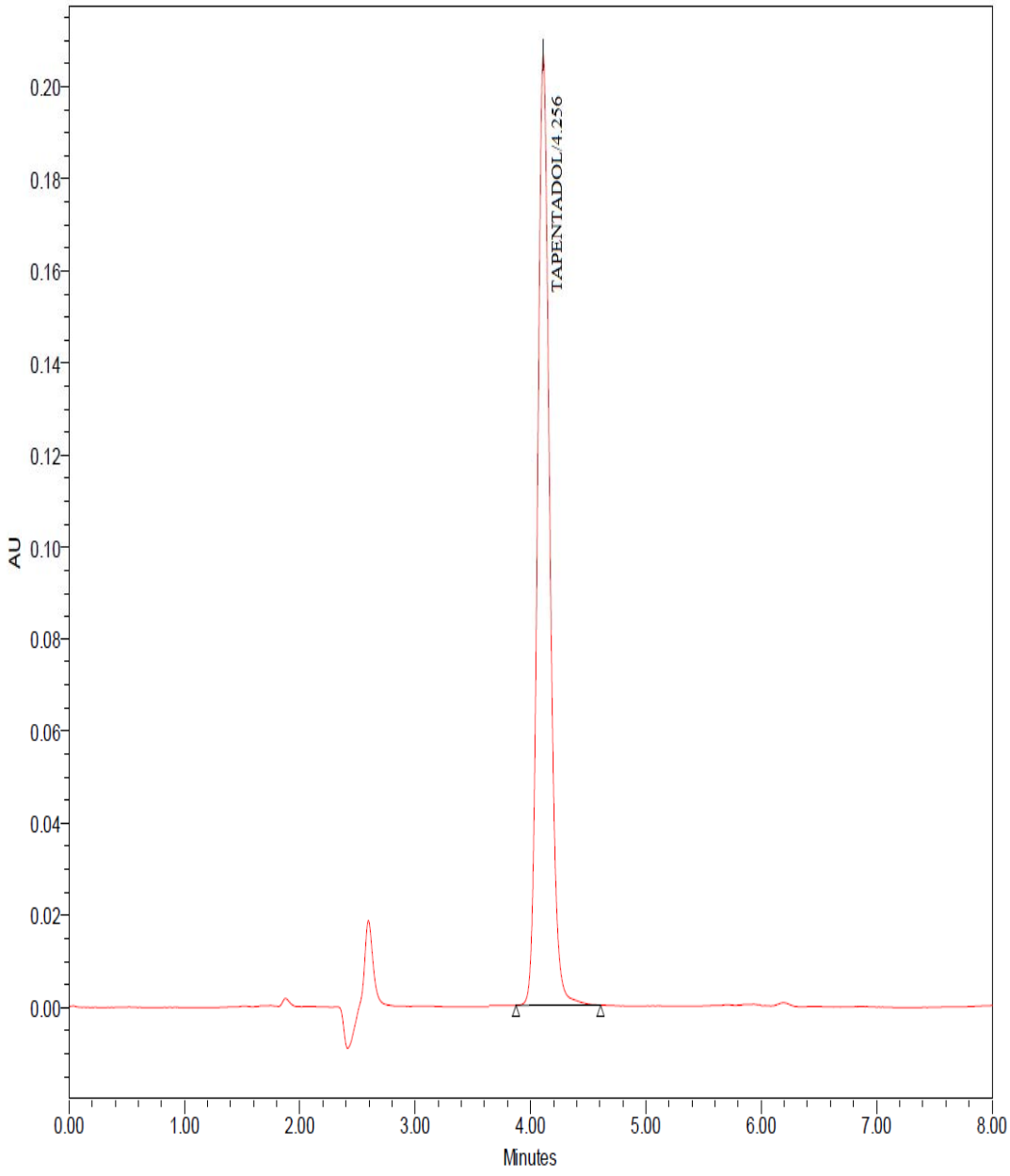


Fig: 10 Chromatogram of Linearity Standard solution of Tapentadol Hydrochloride (100ug/ml)

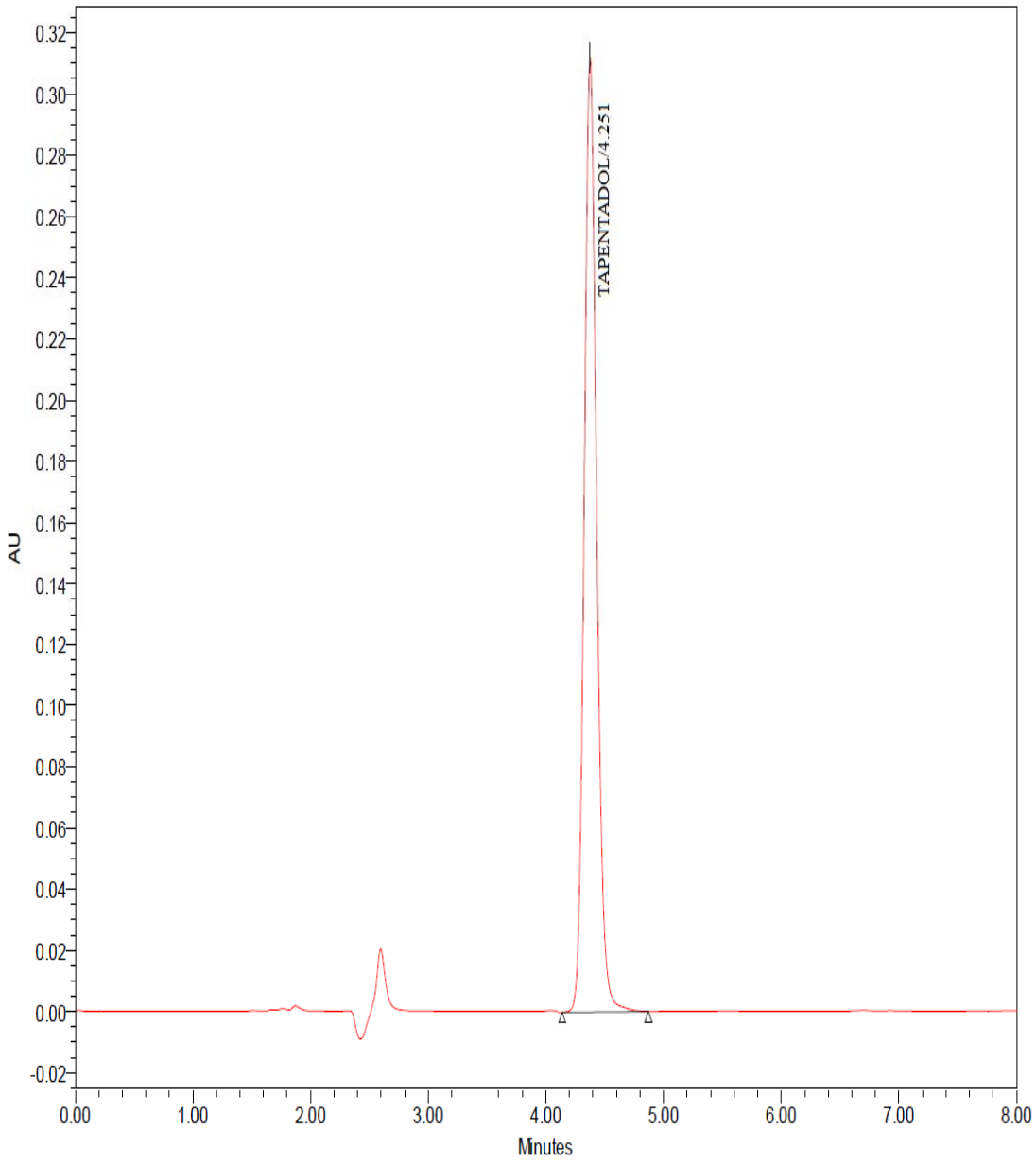


Fig: 11 Chromatogram of Linearity Standard solution of Tapentadol Hydrochloride (125ug/ml)

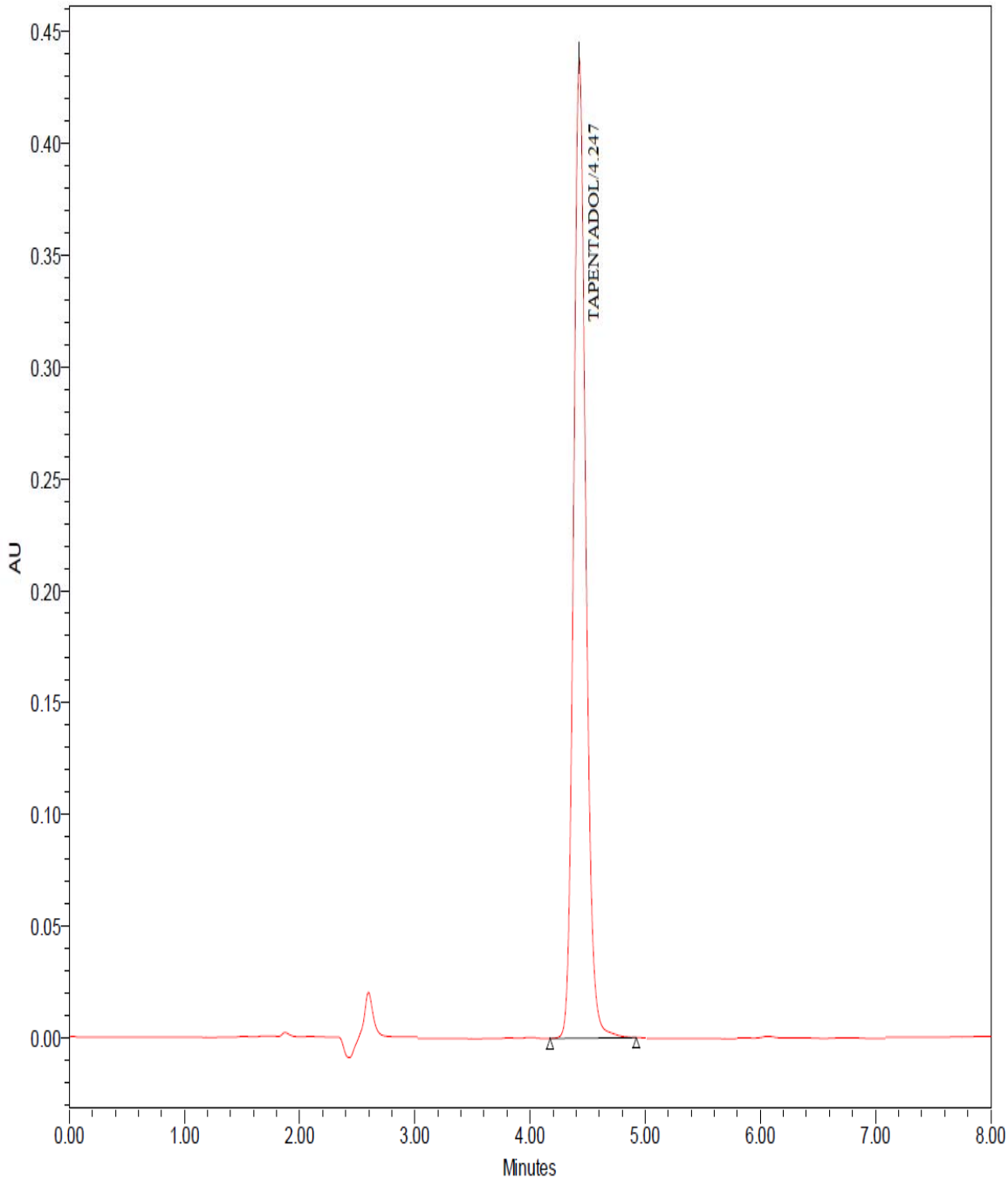


Fig: 12 Chromatogram of Linearity Standard solution of Tapentadol Hydrochloride (150ug/ml)

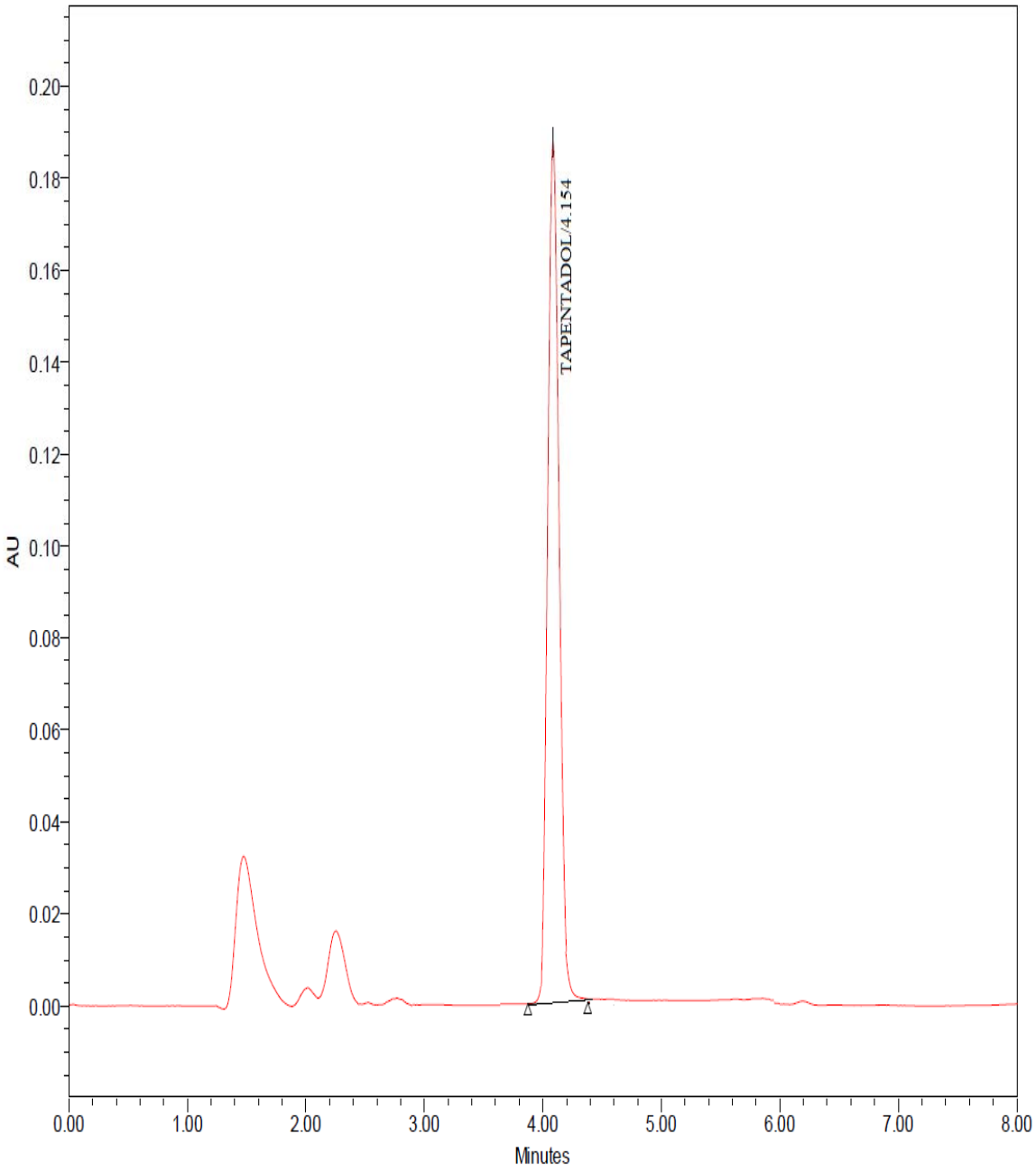


Fig: 13 chromatogram of sample solution

SUMMARY AND CONCLUSION

The working condition for the HPLC method was established for Tapentadol Hydrochloride and then was applied on pharmaceutical dosage forms. A simple Reverse Phase High Performance Liquid Chromatographic method has been developed and subsequently validated.

The separation method was carried out by using a mobile phase consisting of Acetonitrile and potassium dihydrogen phosphate pH 7±0.05 in the ratio 50:50. The detection was carried out by using PDA detector at 215nm. The column was Phenomenex C18 (250 X 4.6mm 5 µ). The flow rate was selected as 1.23ml/min.

The retention time of Tapentadol Hydrochloride was found to be 4.2. The asymmetry factor or tailing factor of Tapentadol Hydrochloride was found to be -1.38, which indicates symmetrical nature of the peak. The number of theoretical plates of Tapentadol Hydrochloride was found to be 2032.49, which indicates the efficient performance of the column. These parameters represent the specificity of the method.

From the linearity studies, specified concentration levels were determined. It was observed that Tapentadol Hydrochloride was linear in the range of 50% to 150% for the target concentration by RP-HPLC. The linearity range of Tapentadol Hydrochloride 50-150µg/ml was found to obey linearity with a correlation coefficient of 0.999.

The validation of the proposed method was verified by system precision and method precision by RP-HPLC. The %RSD of system suitability for Tapentadol Hydrochloride was found to be 0.81.

The validation of the proposed method was verified by recovery studies. The percentage recovery range was found to be satisfied which represent in results. The ruggedness study was also performed.

CONCLUSION:

A HPLC method for Tapentadol Hydrochloride was developed and validated in tablet dosage form as per ICH Guidelines.

Waters 2996 series with PDA Detector and Phenomenex C18 (250x4.6mm, 5µ) column, injection of 20 µl is injected and eluted with the mobile phase of potassium dihydrogen phosphate buffer with pH 7 and Acetonitrile in the ratio 50:50, which was pumped at a flow rate of 1.23 ml at 215nm. The peak of Tapentadol Hydrochloride was found well separated at 4.2 min. The developed method was validated for various parameters as per ICH guidelines like system suitability, accuracy, precision, linearity, specificity, ruggedness, robustness and solution stability.

The analytical method validation of Tapentadol Hydrochloride by RP HPLC was found to be satisfactory and could be used for the routine pharmaceutical analysis of Tapentadol Hydrochloride.

GLOSSARY

1. **Degassing:** The process of removing dissolved gas from the liquid mobile phase before or during use. Degassing is done by heating, by vacuum, or by helium purging.
2. **Drift:** The change in the baseline value over time, expressed mathematically as the slope of the least line squares line fitted to the base line in a specified region chromatogram.
3. **Eluent:** Mobile phase used to perform a chromatographic separation .The liquid that exits through a chromatographic column during a separation.
4. **Equilibration:** The process of bringing a chromatographic solvent (solvent, column, and interactive surfaces) to a thermally and chemically stable state, usually indicated by a drift –free base line.
5. **External Standard:** A separate sample that contains known quantities of the same compounds that are in unknown samples. External standards are used for quantitation by matching the detector response of a component peak to a point on a calibration curve for that component. The calibration curve is generated from a separately processed standard (or set of standards).
6. **Fronting peak:** An asymmetrically shaped chromatographic peak in which the front part of the peak (before the apex) contains more area than the rear of the peak. The asymmetry factor for fronting peak has value less than one. The opposite of fronting peak is a tailing peak
7. **Fused peak:** Two or more no baseline-resolved peaks in a chromatogram that share the same base line, the same base line start and end points, and the same slope and offset.
8. **Gradient Elution:** Also called as solvent programming, a technique for decreasing the separation time by increasing the mobile phase strength over time during a

chromatographic separation. Gradients can be continuous or stepwise. Binary (2-solvent), ternary (3-solvent0 and quaternary (4-solvent) gradients are used routinely in HPLC.

9. **Integration:** The mathematical process of calculating an area such as a chromatographic peak that is bounded in a part or in a whole by a curved line.
10. **Isocratic:** The condition in which the solvent composition, flow rate, and the temperature are constant during a chromatographic run, the condition in which the solvent composition is constant during a chromatographic run.
11. **Linearity:** The condition in which detector response is directly proportional to the concentration or amount of a component over a specified range of component concentrations or amounts .A calibration curve is a straight line when the standard concentrations are within the linear response range of the detector. In chromatography, accurate quantitation requires linearity of the detector response over the range of actual sample concentrations or amounts.
12. **Mobile Phase:** The fluid (gas or liquid) that carries solutes through a chromatographic column. In LC, the liquid that is pumped through the fluid path of the chromatographic system and into which the samples are injected.
13. **Plate count:** A measure of the observed chromatographic resolution based on its equivalency to the number of theoretical plates that would provide the same resolution.
14. **Resolution:** The extent to which a chromatographic column separates components from each other. Mathematically defined, resolution is the difference between the peak retention time of a selected peak and the peak preceding its multiplied by a constant of 1.18,then dived by the sum of the peak widths at 50%of peak height. It is used to monitor the separation of eluting components and to establish system efficiency.

15. **Retention Time:** The time that elapses between the injection of a sample and the appearance of the peak maximum of a component in a sample.
16. **RT ratio:** The Retention Time of a component divided by the retention time of its reference peak.
17. **System suitability:** An application that applies a set of standard criteria to test if an entire chromatographic system and the methodology are working within acceptable limits. Empower software bases the system suitability tests on standard laboratory calculations, including United States Pharmacopoeia (USP) guidelines and calculations. Empower software produces reports showing statistical accuracy and reproducibility of the chromatographic system data.
18. **Tailing Factor:** A measure of peak symmetry, where a Symmetrical peak has a tailing factor of 1.As Tailing increases, peak symmetry increases for System suitability, the tailing factor is the width of the peak at 5% height, divided by two times the distance from the peak maximum to the leading edge of the peak (where the distance is measured at point 5 % of the peak height from the base line).
19. **Capacity factor:** A chromatographic parameter that measures retention time of a sample molecule relative to the column dead volume.
20. **Acceptance Criteria:** Numerical Limits, ranges, or other suitable for acceptance of the results of analytical procedures.
21. **Detection Limit**: The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated as an exact value.

22. **Drug Product**: A finished dosage form, for example, a capsule, tablet, or solution that contains a drug substance, but not necessarily, in association with one or more other ingredients.
23. **Drug Substance/Active Ingredient**: An active ingredient that is intended to furnish pharmacological activity or other direct effect the structure or any function of the human body. The active ingredient does not include intermediates used in the synthesis of such ingredient. The term includes those components that may undergo chemical change in the manufacture of the drug product and be present in the drug product in a modified form intended to furnish the specified activity or effect.
24. **Reagent:** For analytical procedures, any substance used in a reaction for the purpose of detecting, measuring, examining, or analyzing other substances.
25. **Specification**: The quality standards (i.e., tests, analytical procedures, and acceptance criteria) provided in an approved application to confirm the quality of the drug substances, drug products, intermediates, raw materials, reagents, and other components including container closure systems, and in-process materials.
26. **Spiking**: The addition of a small known amount of a known compound to a standard, sample, or placebo, typically for the purpose of confirming the performance of an analytical procedure or the calibration of an instrument.
27. **Working Standard**: A standard that is qualified against and used instead of the reference standard (also known as in-house or secondary standard).

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